CYTOKINES AS BIOMARKERS IN ASTHMA
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Asthma is a lung disease characterized by wide variations in airflow over short periods of time. Exacerbations of asthma can be accompanied by symptoms of chest tightness, shortness of breath and wheezing; airway inflammation characterized by an influx of eosinophils and/or neutrophils; and the expression of pro-inflammatory cytokines in the airway. There is strong evidence supporting a central role for the T cell in asthma. In atopic asthma, T cells are documented components of the late-phase response to inhaled allergen, driving airway inflammation, mucus hypersecretion, and bronchoconstriction through the release of cytokines and other mediators. T cells have also been shown to produce inflammatory cytokines in response to allergen in nonatopic asthmatics, indicating a potential role in mediating disease in this phenotype. In both atopic and nonatopic asthma, aberrant T cell responses to allergen may drive the infiltration of neutrophils and eosinophils into the airway through the production of pro-inflammatory cytokines, leading to exacerbations of disease. This project has investigated the role of several T cell cytokines in driving disease and acting as biomarkers in asthma: interleukin-5, interleukin-17A, interleukin-23, interleukin-10, and interferon-γ. We have measured allergen-induced cytokine production by peripheral blood mononuclear cells (PBMCs) and examined its ability to distinguish between different asthma phenotypes: asthma vs normal, atopic vs nonatopic asthma, eosinophilic bronchitis vs noneosinophilic bronchitis, and neutrophilic vs nonneutrophilic bronchitis. Our data shows that allergen-induced peripheral blood mononuclear cell responses to allergen are not good biomarkers of disease in asthma. No differences in PBMC cytokine production are seen in patients with asthma, compared with normal controls, or between patients with different asthmatic phenotypes. It is not possible to determine a patient’s disease state, atopic status, or type of bronchitis by examining their PBMC cytokine responses to allergen.
DEDICATION

I would like to dedicate my thesis to Dr. Frederick Hargreave, a physician and researcher at the Firestone Institute for Respiratory Health at St. Joseph’s Hospital in Hamilton. Dr. Hargreave’s contributions to the field of asthma research have shaped the understanding and treatment of this disease and have made my project possible. He has been an invaluable member of my supervisory committee, and without his knowledge and encouragement this project could not have been successfully completed. Dr. Hargreave passed away suddenly just three weeks before the writing of this thesis. His dedication to his patients and his passion for his work continue to inspire me.
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INTRODUCTION

ASTHMA

Asthma is most commonly defined as a unique disease entity characterized by airway inflammation, reversible airway obstruction, and airway hyperresponsiveness leading to symptoms of wheezing, difficulty breathing, coughing, and chest tightness. This airway disease is estimated to affect 300 million people worldwide, causing 250,000 deaths annually. It results in a decreased quality of life for the affected individual, and places a burden on society as a whole due to elevated healthcare costs and decreased productivity of asthmatic individuals.

Despite its widespread use, the above definition of asthma is a controversial one. A single genetic or environmental cause of asthma has not been identified, and this descriptive definition fails to differentiate asthma from other lung diseases and accurately represent the disease heterogeneity seen between and within asthmatic patients.

For the purposes of this project, asthma refers to an airway abnormality that is characterized by wide variations in airflow over short periods of time. These variations in airflow may occur spontaneously, in response to a stimulus (i.e. bronchoconstriction following the inhalation of an allergen) or as a result of treatment (i.e. the reversal of bronchoconstriction after inhalation of a rapid-acting β-agonist). Patients with asthma may also express other components of airway disease, including airway inflammation, obstruction, and remodeling. The presence, nature, and severity of these disease components can vary between and within asthmatic patients over time.

Airway inflammation occurs when an individual’s immune system reacts to stimuli including allergens, pollutants, microbes, and viruses, leading to an influx of inflammatory cells into the airway. Airway obstruction is the result of mucus hypersecretion that blocks airflow and/or the constriction of airway smooth muscle. Remodeling refers to characteristic structural changes of the airway, involving collagen deposition under the epithelium, thickening of the airway smooth muscle, and an increased number of mucus-secreting goblet cells. Remodeling in asthma occurs as a consequence of repeated damage to the airway, followed by inadequate repair. This damage may be the result of allergic reactions, poor clearance of viral infections, and reduced antioxidant defense mechanisms. It is coupled with an altered repair process, in which the airway responds to damage by activating pro-inflammatory pathways, as opposed to appropriate repair pathways.

Asthma is most commonly treated using rapid-acting and long-acting β-agonists, and inhaled corticosteroids. β-agonists act to reverse bronchoconstriction by relaxing airway smooth muscle and inhaled corticosteroids are anti-inflammatory drugs used to reduce chronic airway inflammation. In cases of severe asthma exacerbations, oral steroids such as prednisone may also be employed.
ATOPIC AND NONATOPIC ASTHMA

Asthma has traditionally been classified into three categories: atopic, nonatopic, and occupational. Atopic asthma occurs in individuals whose disease is mediated by IgE antibodies produced against allergens the individual has been sensitized to. Nonatopic asthma is non-IgE mediated and is considered to occur in individuals who are nonallergic. Occupational asthma occurs after individuals have been exposed to specific proteins or small molecular weight chemicals in their workplace. The importance of IgE in this category of asthma has not yet been established.

In approximately half of asthmatic patients, symptoms are triggered by an allergic response to an identifiable antigen. These patients are classified as having atopic asthma, and the allergens that trigger their asthma are identified using a skin-prick test. A positive skin-prick test indicates that the B-lymphocytes of the patient are producing IgE antibodies against a particular allergen, and the patient is therefore classified as being allergic to that material. Inhalation of this allergen results in the cross-linking of allergen-specific IgE antibodies bound to FceRI receptors on the surface of mast cells. Receptor activation signals mast cells to degranulate, releasing inflammatory mediators such as histamine, leukotrienes, prostaglandins and inflammatory cytokines like IL-4, IL-5 and IL-13. This release of pro-inflammatory granules leads to vasostriction, smooth muscle contraction, and an influx of inflammatory cells into the airway. Once a patient is aware of his or her allergies, steps can be taken to avoid or treat the allergy, resulting in a reduction of asthma symptoms.

Asthmatic patients who do not test positive for any allergies when the skin-prick test is administered, and have no clinical history of allergic disease, are classified as nonatopic asthmatics. It is assumed that their asthma is triggered by something other than a typical allergic response mediated by allergen-specific IgE antibodies and mast cell degranulation.

Asthma in nonatopic patients is remarkably similar to asthma in patients who have typical allergic asthma. The two patient groups experience wide variations in airflow over short periods of time and exhibit many of the same components of airway disease: similar symptoms of chest tightness, shortness of breath and wheezing; airway inflammation characterized by an influx of eosinophils and/or neutrophils; and expression of pro-inflammatory cytokines in the airway, such as IL-4 and IL-5. These are interesting similarities between what have traditionally been classified as two separate disorders: atopic asthma and nonatopic asthma.

These striking similarities between the two types of asthma have prompted us to ask the question: could asthma in nonatopic patients actually be triggered by exposure to allergens? These sensitivities have not been identified by a conventional skin-prick test, and may not be IgE-mediated, but may be driven by other immune pathways. Potential allergen-associated mechanisms of disease at work in nonatopic asthma will be discussed further in a later section of this report.
EOSINOPHILS AND NEUTROPHILS IN ASTHMA

Airway inflammation in asthma can be classified according to the predominant inflammatory cell type present in a patient’s sputum: neutrophils, eosinophils, both, or neither. This inflammatory infiltrate will vary over time with changes in the patient’s environment and/or treatment.26

Eosinophils

Eosinophils are granular leukocytes that play an important role in the defense against parasitic infections. They have the ability to bind IgE-coated parasitic worms, via Fcε receptors, and release toxic granule proteins and free radicals that neutralize the parasite. Eosinophils also play an important role in the pathogenesis of allergy and asthma. When these cells are recruited and activated inappropriately, the release of their toxic granules and cytokines leads to tissue damage and inflammation.39

Eosinophils originate in the bone marrow as CD34+ precursors. Though several cytokines, including IL-3 and GM-CSF, are important in the early stages of CD34+ commitment to eosinophil lineage, IL-5 is the main cytokine driving eosinophil maturation and recruitment to the airway.70

Once in the airway, eosinophils secrete a host of harmful granules and cytokines. Eosinophil collagenase, eosinophil peroxidase, and major basic protein (MBP) are toxic to mammalian cells. MBP also triggers histamine release from mast cells, inducing vasodilation and smooth muscle contraction.13 Leukotrienes instigate smooth muscle contraction, increase vascular permeability and increase mucus secretion. IL-8, IL-3, IL-5, and GM-CSF attract neutrophils, macrophages, and other eosinophils, resulting in a cascade of pro-inflammatory signals.39

Though the significance of IL-5 and eosinophils in asthma has been debated,21 two studies in particular have demonstrated their importance in a subset of asthmatic patients. Unlike previous reports,46 these studies focused on patients whose disease is driven by persistent airway eosinophilia.

A 2009 study published in The New England Journal of Medicine examined a rare group of asthmatic patients who have sputum eosinophilia and airway symptoms despite continued treatment with prednisone and high-dose corticosteroids.60 Patients were treated with mepolizumab, a monoclonal antibody against IL-5, and prednisone sparing, asthma symptoms, blood and sputum eosinophil levels, FEV1 and frequency of asthma exacerbations were assessed. Among the 10 patients who were treated with a placebo, there were 12 asthma exacerbations, 9 with sputum eosinophilia at the time of exacerbation. Patients in this group were able to reduce their prednisone dose by approximately 47.7% of the maximum possible reduction (from a mean of 10.7 mg to a mean of 6.4 mg), though this reduction was accompanied by an increase in blood and sputum eosinophil levels. Among the 9 patients treated with mepolizumab, there was 1 exacerbation, which was not accompanied by sputum eosinophilia (instead it was accompanied by sputum neutrophilia). These patients were able to reduce their prednisone dose by approximately 83.8% of the maximum possible reduction (from a mean of 11.9 mg to a mean of 3.9 mg), and this was not accompanied by a significant increase in
blood and sputum eosinophil levels. Patients in the mepolizumab group also saw improvements in quality of life and FEV\textsubscript{1} values, which were maintained for up to 8 weeks after treatment with no increase in prednisone. It was concluded that mepolizumab treatment did provide clinical benefit in this group of asthmatic patients, allowing for a reduction in prednisone dose and a decreased risk of asthma exacerbations.

A similar article was published in the same issue of *The New England Journal of Medicine*\textsuperscript{23}. This study examined the effect of mepolizumab on the frequency of asthma exacerbations in patients with refractory eosinophilic asthma and a history of recurrent severe exacerbations. Patients who received mepolizumab treatment experienced significantly fewer exacerbations than those in the placebo group and reported an increased quality of life. Decreased airway wall area and thickness were also seen in the mepolizumab group. A similar effect has been seen in a mouse model of allergic asthma – eosinophil-deficient mice showed a significant protection from collagen deposition in the airway and increases in airway smooth muscle\textsuperscript{35}.

These studies demonstrate the importance of IL-5 and eosinophils in asthma pathogenesis, and underscore the need to understand and treat asthma as a heterogeneous disease. It is necessary to recognize the impact that the eosinophil has on a subset of asthmatics prone to eosinophilic bronchitis. Targeting this cell type can help improve disease in these patients, and it has been demonstrated that using sputum cell counts to guide corticosteroid treatment can be an effective method for controlling asthma exacerbations\textsuperscript{40}.

**Neutrophils**

Neutrophils are granulocytes that play an important role in the immune response to bacterial infection. They act as phagocytes to engulf and destroy microbes with toxic granules, and also release a host of bactericidal agents that are effective at killing bacteria, but are harmful to host cells. Neutrophils are recruited to the airway through chemotactic interactions of IL-8 and Gro-\alpha with their CXCR2 receptor\textsuperscript{39}.

In asthma, IL-17A secreted by Th17 cells stimulates airway epithelial cells to produce the pro-neutrophilic chemokines IL-8 and Gro-\alpha\textsuperscript{78}. Once recruited to the airway, neutrophils produce a number of compounds with damaging potential. Neutrophil elastase degrades elastin (a structural component of the airway) and induces mucus hypersecretion from goblet cells. Matrix metalloproteinases (MMP-8 and MMP-9) degrade extracellular matrix components, and reactive oxygen species have been shown to induce nonspecific airway hyperresponsiveness\textsuperscript{3}.

Neutrophils have come to be associated with severe asthma and asthma-related death. One study found that patients who suffered short duration fatal attacks of asthma (dying within two hours of attack onset) had increased levels of neutrophils in the large and small airways, compared to patients who suffered long duration fatal attacks of asthma (dying within five hours of attack onset)\textsuperscript{12}. Another reported that patients intubated for acute severe asthma displayed neutrophil counts in tracheal aspirate 10x higher than normal, and neutrophil number correlated with duration of intubation. The pro-neutrophilic cytokine IL-8 was present at levels 19x higher than normal, and correlated with the number of
neutrophils and the duration of mechanical ventilation of the patient. Sputum neutrophil counts are significantly increased in severe asthma when compared to mild asthma and normal controls, and asthmatic patients with severe disease exhibit the highest levels of IL-8 and neutrophil myeloperoxidase. A higher sputum total neutrophil count has been associated with lower postbronchodilator FEV₁, suggesting a role for neutrophils in persistent airflow limitation. None of these studies found eosinophils and their associated cytokines to be similarly implicated, and thus have prompted speculation that neutrophilic asthma may be a distinct asthma phenotype, associated with more severe disease.

It is important to note that none of these studies have accounted for the possibility of undetected respiratory infection. Since neutrophils play a key role in the response to pathogenic microbes and associated asthma exacerbations, lung infection could account for the influx of neutrophils seen in patients with neutrophil-associated exacerbations of asthma and asthma-related death. Another important aspect to consider when discussing neutrophilic asthma exacerbations is inhaled corticosteroid treatment. Corticosteroids effectively suppress eosinophilia in asthma, but can increase neutrophils and neutrophil-associated cytokines in the airway. These medications also serve to suppress the immune system of the patient, potentially resulting in a greater susceptibility to microbial infection. Thus, it is possible that some neutrophilic-associated asthma exacerbations are a consequence of the effects of corticosteroid treatment.

**MECHANISM OF DISEASE**

Since asthma is characterized by variations in airflow over time, asthmatic patients often experience periods where their disease is well controlled, followed by exacerbations involving bronchoconstriction and airway symptoms. Exacerbations of asthma are often triggered by exposure to environmental allergens or pathogens, but they can also occur spontaneously or in response to other stimuli such as exercise or cold air.

During a typical allergic exacerbation, effector cells of the innate and adaptive immune systems, as well as airway structural cells, play an integral role in the pathogenesis of asthma. These exacerbations can be broken down into an acute response and a late-phase response, both of which are mediated by complex interactions between antibodies, immune cells, airway epithelium, and airway smooth muscle.

**Acute Response**

The acute response is an immediate reaction that occurs within minutes of allergen inhalation. It is initiated by inhaled allergen crossing the airway epithelial barrier and directly activating mast cells by cross-linking allergen-specific IgE antibodies bound to FceRI cell-surface receptors. In asthmatic patients, the airway epithelial barrier displays increased permeability, characterized by a loss of columnar cells and disruption of tight junctions between cells. This decreased epithelial integrity allows for greater infiltration of allergens and other irritants into asthmatic airways.
Mast cells are the primary cells involved in the acute response. They are granulocytes that are recruited to the airway surface by stem-cell factor secreted by airway epithelial cells as well as IL-8 and CXCL-10 secreted by airway smooth muscle cells. Cross-linking of allergen-specific IgE bound to FcεRI cell-surface receptors stimulates mast cell degranulation, releasing histamine, heparin, proteases, cytokines, matrix metalloproteinases, eicosanoids, and leukotrienes. These inflammatory mediators stimulate smooth muscle contraction, microvascular permeability, chemotaxis of other inflammatory cells, and airway remodeling.

Late-phase Response

The late-phase response occurs within 3-4 hours after initial exposure to allergen, and may persist for several days. It is characterized by the infiltration of Th2 cells, neutrophils, and eosinophils into the airway. The process begins when immature dendritic cells (DCs) take up inhaled allergen by extending cellular projections through the airway epithelium and into the lumen. Thymic stromal lymphopoietin (TSLP) is produced by airway epithelial cells in response to allergen, and stimulates these immature DCs to mature and migrate to the draining lymph nodes. This migration to the lymph nodes is guided by the chemotactic interaction of the CCR7 receptor of DCs with its ligands CCL19 and CCL21. DCs upregulate the co-stimulatory molecule OX40L and MHC class II, onto which they load processed allergen in preparation for presentation to T cells. In the draining lymph nodes, DCs present antigen to naïve CD4+ T cells on MHC class II and deliver a co-stimulatory activation signal through interactions between OX40L expressed on DC cell membranes and OX40 expressed on T cell membranes. Delivered together, these two signals stimulate T cells to start producing interleukin-4 (IL-4), polarizing them to become allergen-specific inflammatory Th2 cells by inducing the transcription factor STAT6 to activate the expression of the GATA3 transcription factor. These newly primed Th2 cells receive chemotactic signals through their CCR4 receptors to migrate to the airway. They respond to a number of chemokines, including CCL17 (TARC) and CCL22 (MDC) produced by TSLP-stimulated DCs.

Once in the airway, Th2 cells secrete a host of inflammatory cytokines important in asthma. IL-4 and IL-13 stimulate B cells to produce allergen-specific IgE antibodies. IL-3, IL-4, IL-9, and IL-13 recruit mast cells and basophils. IL-5 stimulates the maturation and recruitment of eosinophils. IL-13 induces mucus hypersecretion from goblet cells and airway smooth muscle contraction, leading to the airway obstruction and subsequent reduction in airflow characteristic of asthma.

The T cell-mediated infiltration of eosinophils and neutrophils into the airway plays a key role in asthma exacerbations. As mentioned above, eosinophils and neutrophils produce a host of inflammatory mediators and other molecules that promote airway narrowing, tissue damage and airway remodeling.
**Other Important Contributors to Asthma Pathogenesis**

Aside from producing TSLP, which plays an important role in DC-mediated activation of Th2 cells, airway epithelial cells contribute to asthma pathogenesis in other ways. In response to allergen exposure, the airway epithelium produces granulocyte-macrophage colony-stimulating factor (GM-CSF), which stimulates the proliferation and differentiation of precursors of neutrophils, eosinophils, and monocytes. This cytokine also enhances cell-surface adhesion proteins on mature eosinophils and neutrophils that could lead to their accumulation in the airway, and increases their generation of reactive oxygen intermediates which cause tissue damage and remodeling.

Airway smooth muscle cells and mucus-producing goblet cells are increased in asthma and are both important contributors to reduced airflow. Constriction of airway smooth muscle narrows the airway, while mucus hypersecretion by goblet cells obstructs the airway. Airway smooth muscle cells can also secrete pro-inflammatory mediators that promote mast cell recruitment and proliferation and neutrophil recruitment.

Th17 cells are a CD4+ T cell subset that produces IL-17A, a cytokine known to stimulate airway epithelium, bronchial fibroblasts, and airway smooth muscle cells to produce the pro-neutrophilic cytokines IL-8 and Gro-α. Th17 cells develop and mature in response to IL-6 and IL-23 produced by activated dendritic cells, and play a role in driving exacerbations of asthma associated with an influx of neutrophils into the airway.

**Alternate Mechanisms of Disease**

The mechanisms described above are known to drive disease in atopic asthma, while those at work in nonatopic asthma remain unclear. Since nonatopic asthma may account for up to half of all asthma cases, it is important to discover the underlying driving forces of disease in these patients. The following alternate mechanisms have been proposed to help explain the pathogenesis of nonatopic asthma.

**Local IgE Production in the Lung**

Although patients with nonatopic asthma are classified as such based on the absence of detectable IgE production, studies have shown that IgE may in fact play a role in driving their airway disease locally in the lung.

Gould et al have demonstrated that class switching from IgM/IgG/IgA to IgE in the bronchial mucosa occurs in both atopic asthmatics and nonatopic asthmatics. Both groups of patients express mRNA coding for: 1) ε circle transcripts (Iε-Cμ CT and Iε-Cγ CT), which indicate DNA recombination is occurring at the heavy-chain locus to produce the ε heavy-chain characteristic of IgE and 2) the ε heavy-chain of IgE itself. The presence of both of these mRNA transcripts indicates that class switching to IgE does occur in the airways of nonatopic asthmatic patients.

Burney and colleagues have demonstrated that nonatopic asthmatic patients can in fact produce IgE in response to airborne allergen. The study asked 297 patients using bronchodilators, half of which were nonatopic, to report any acute...
respiratory events. Over the coming months, small particles were collected on the roof of their clinic using a high volume sampler. These were assumed to be representative of small particles and aeroallergens present in the air around the clinic. When a patient reported an exacerbation, blood was drawn. Particles collected the weekend before a patient’s exacerbation were assessed for their ability to bind IgE in the patient’s serum. That same ability was assessed for particles collected on a control weekend (2-3 weeks before or after the reported exacerbation). Exacerbations were associated with a 25% increase in IgE binding to particles collected on the weekend before the exacerbation compared to the control weekend. This was seen in patients with and without positive skin-prick tests to grass or tree pollens, suggesting that airborne allergen may be an important trigger of IgE-mediated asthma exacerbations, even in nonatopic patients. This mechanism may be local and specific to the lung or the inhalation route, as no reaction was seen with the skin-prick test\textsuperscript{10}. IL-4 and IL-13 mRNA have been found to be elevated in the bronchial biopsies of nonatopic asthmatic patients\textsuperscript{34}, indicative of a cytokine milieu in nonatopic asthmatic airways that is conducive to IgE production by B cells.

\textit{IgE-independent T cell-mediated Response to Allergen}

Evidence of T cell mediated responses to allergen, in the absence IgE involvement, has been reported in the literature. This suggests that the late phase of an ‘allergic’ response may occur in the absence of the acute response and without the corresponding IgE production traditionally used to identify trigger allergens in asthmatic patients.

Haselden \textit{et al} have demonstrated that intradermal administration of peptides derived from cat allergen can elicit late phase asthmatic responses in cat-allergic asthmatics. These peptides did not cross-link IgE when tested \textit{in vitro}, or elicit the early wheal and flare cutaneous response that is usually associated with an allergic response to antigen. However, 2-3 hours following peptide administration, 9 of 40 subjects experienced a decline in FEV\textsubscript{1}, as well as chest tightness and wheezing\textsuperscript{28}.

Mori \textit{et al} have demonstrated that peripheral blood mononuclear cells from nonatopic asthmatic patients can produce IL-5 when incubated with \textit{Candida albicans} extract\textsuperscript{57}. When these patients were given an inhalation challenge of \textit{Candida albicans} acid protease they experienced a late phase asthmatic response characterized by a 45% drop in FEV\textsubscript{1} 6 hours after challenge. A skin-prick challenge also elicited a late phase skin response 24 hours after the test was administered\textsuperscript{56}.

\textbf{PROJECT RATIONALE}

There is strong evidence supporting a central role for the T cell in asthma. In atopic asthma, T cells are documented components of the late-phase response to inhaled allergen, driving airway inflammation, mucus hypersecretion, and bronchoconstriction through the release of cytokines and other mediators. T cells have also been shown to produce inflammatory cytokines in response to allergen in nonatopic asthmatics, indicating a potential role in mediating disease in this phenotype. In both atopic and nonatopic asthma, aberrant T cell responses to
allergen may drive the infiltration of neutrophils and eosinophils into the airway through the production of pro-inflammatory cytokines, leading to exacerbations of disease.

This project will investigate allergen-induced T cell cytokines as biomarkers of asthma, both atopic and nonatopic. For this investigation, it will be important to consider the type of airway inflammation characteristic of each patient. We hypothesize that pro-eosinophilic cytokines will characterize the allergen-induced T cell responses of patients with a history of eosinophilic bronchitis, and that pro-neutrophilic cytokines will characterize the allergen-induced T cell responses of patients with a history of neutrophilic bronchitis.

**Cytokine Selection**

T cell cytokines were chosen based on an association with asthma and their potential ability to either drive disease by instigating airway inflammation (IL-5, IL-17A, and IL-23) or protect against disease by countering inappropriate immune responses (IL-10 and IFN-γ).

**Interleukin-5**

IL-5 is predominantly produced by Th2 cells and is the main cytokine responsible for driving the maturation and recruitment of eosinophils to the airway. IL-5 protein and mRNA has been detected in the bronchial mucosa of both atopic and nonatopic asthmatic patients.

Animal models have demonstrated that transgenic overexpression of IL-5 results in systemic eosinophilia, and that constitutive IL-5 expression in the lung causes the accumulation of eosinophils in the airways and airway hyperresponsiveness to methacholine. They have also shown that using an anti-IL-5 monoclonal antibody to neutralize IL-5 inhibits antigen-induced airway eosinophilia and airway hyperresponsiveness.

IL-5 mRNA expression is increased in bronchial biopsies from asthmatic patients in comparison to normal controls. In addition, asthmatic patients expressing IL-5 mRNA in the airway show a significant increase in the number of activated T cells and activated eosinophils when compared to IL-5 mRNA-asthmatic patients. IL-5 mRNA expression in bronchial biopsies has also been shown to correlate with symptom severity and airway hyperresponsiveness in atopic asthma, indicating that it may play an important role in driving disease. In comparison to normal controls, IL-5 production from CD4+ T cells in response to non-specific stimulation is enhanced in both atopic and nonatopic asthmatics. This cytokine also plays a role in the asthmatic response to allergen, as allergen-specific T cells isolated from mite-allergic atopic asthmatic patients produce IL-5 in response to dust mite.

**Interleukin-17A**

IL-17A is principally produced by CD4+ Th17 cells, but other cell types, such as eosinophils, have been shown to produce this cytokine as well. Th17 cells are important in host defense against bacteria and fungi, and have also been implicated in inflammatory disease. Transfer of Th17 cells into mice has been
shown to result in experimental autoimmune encephalitis, which worsens with increasing numbers of Th17 cells. Treatment with an IL-17A neutralizing antibody affords partial protection against this inflammation.

In the lung, IL-17A acts on bronchial epithelial cells to induce the production of IL-8 and Gro-α, resulting in the recruitment of neutrophils to the airway. Animal models have demonstrated that intratracheal administration of IL-17A in rats induced neutrophil recruitment and activation in the airway. In OVA-challenged mice, the adoptive transfer of Th17 cells resulted in neutrophil accumulation in the lung and airway hyperresponsiveness to methacholine that was not reversible with steroid administration. This response was not seen in IL-17RA knockout mice, indicating that IL-17A was the driving force.

Increased levels of IL-17A are present in the sputum and bronchoalveolar lavage of asthmatic patients, and levels of IL-17A in sputum correlates with airway hyperresponsiveness to methacholine. Allergic asthmatics display elevated plasma IL-17 in comparison to normal controls, and their T cells produce IL-17A following allergen challenge. Activated peripheral blood mononuclear cells from atopic asthmatics display higher levels of RORγt, the key transcription factor in controlling the differentiation of Th17 cells.

**INTERLEUKIN-23**

IL-23 is a cytokine produced by dendritic cells and is considered to act “upstream” to IL-17A in the process of neutrophil recruitment, as it is integral for the maintenance and survival of Th17 cells. IL-23 is a member of the IL-12 cytokine family, sharing a p40 subunit with IL-12 in addition to having its own unique p19 subunit.

Animal models have demonstrated a role for IL-23 in asthma. IL-23 and IL-23R mRNA are upregulated in the lung following allergen challenge, and production of IL-23 following allergen uptake by dendritic cells is linked to airway hyperresponsiveness in mice.

IL-23 has also been associated with increased airway eosinophilia in animal models, though the mechanism behind this phenomenon is currently unknown. In a mouse model of allergic asthma, transgenic overexpression of IL-23R resulted in increased eosinophil infiltration of the airway and production of IL-4, IL-5, and IL-13. Another group demonstrated that forced overexpression of IL-23 in mice leads to recruitment of both neutrophils and eosinophils to the lung, followed by the production of Th2 cytokines (IL-5 and IL-13), IL-17A, TNF-α, goblet cell hyperplasia, and airway hyperresponsiveness. Silencing IL-23 expression in the lung has been shown to significantly reduce the influx of neutrophils and eosinophils into the lungs of mice following allergen challenge.

Compared with normal controls, IL-23 has been found to be elevated in the peripheral blood plasma of atopic asthmatics and correlates positively with plasma IL-17A levels. Activated peripheral blood mononuclear cells from these patients produced significantly higher levels of IL-23 than controls.
INTERLEUKIN-10

In atopy and asthma, the balance between inflammatory T cells and regulatory T cells is skewed towards the pro-inflammatory type. IL-10 is produced by a population of CD4+ T regulatory cells, some of which express the surface protein CD25 and/or the transcription factor FOXP3. These cells are responsible for suppressing the inflammatory response and maintaining tolerance to self-antigens and harmless environmental antigens. IL-10 acts on antigen presenting cells to dampen their activation of T cells, inhibits mast cells and eosinophils, and promotes IgG4 production, an antibody thought to counter the effects of IgE.

In a mouse model of allergic asthma, the adoptive transfer of allergen-specific CD4+CD25hi T cells increased expression of IL-10 and reduced airway hyperresponsiveness and Th2 inflammation. These effects were ameliorated by the administration of an anti-IL10 mAb. The depletion of CD4+CD25+ regulatory T cells in a dust mite-allergic mouse model increased airway hyperresponsiveness, airway eosinophilia, IgE, and IL-5 and IL-13 production by Th2 cells. Dendritic cells from these mice demonstrated elevated expression of MHC II, CD80, and CD86, accompanied by an increased ability to stimulate T cell proliferation and Th2 cytokine production. An allergic model of IL-10 knockout mice exhibited increased IL-5 in bronchoalveolar lavage and eosinophilic inflammation of the airway.

IL-10 was found to be the predominant cytokine produced by allergen-specific T cells from healthy volunteers, while allergen-specific T cells from atopic individuals produced high IL-4. CD4+CD25+ T cells from atopic donors have an impaired ability to suppress allergen-specific T cell proliferation and IL-5 production, and CD4+CD25+ T cell numbers and function are reduced in the bronchoalveolar lavage of pediatric patients with asthma.

INTERFERON-γ

IFN-γ, the principal cytokine involved in the Th1 response, is produced by CD4+ and CD8+ T cells. Several studies have demonstrated its importance in the resolution of airway inflammation.

Animal models have shown that mice lacking the IFN-γ receptor experience prolonged airway eosinophilia following allergen inhalation. Mice without T-bet, the Th1 transcription factor, display spontaneous features of airway disease, including airway hyperresponsiveness and an infiltration of Th2 cells and eosinophils into the airway. In another model, administration of IFN-γ prevented the accumulation of T cells and eosinophils in the airway following antigen challenge in sensitized mice, while pretreatment with an anti-IFN-γ mAb had the opposite effect, increasing the infiltration of these cells into the airway.

Expression of the Th1 transcription factor T-bet is reduced in the airways of asthmatic patients, while expression of the Th2 transcription factor GATA3 is increased. Serum levels of IFN-γ are significantly decreased in children with asthma, and a two-year study of asthmatic children demonstrated that a decline in lung function, assessed by FEV1 values, correlated with a progressive decrease in
IFN-γ production by peripheral blood mononuclear cells\textsuperscript{74}. The frequency of IFN-γ\textsuperscript{+}CD4\textsuperscript{+} T cells is decreased in the peripheral blood of allergic asthmatics, while the frequency of IL-4\textsuperscript{+}CD4\textsuperscript{+} and IL-17\textsuperscript{+}CD4\textsuperscript{+} T cells is increased\textsuperscript{83}.

**HYPOTHESIS**

Allergen-induced T cell cytokines will act as biomarkers of disease in asthma. In response to allergen, both atopic and nonatopic asthmatic patients will exhibit PBMC activation and cytokine production characteristic of Th2 and Th17 inflammation.

**PROJECT OBJECTIVES**

1. To investigate differences in PBMC responses to allergen between asthmatic patients and normal controls:
   a) Do PBMCs from asthmatic patients show greater activation in response to allergen in comparison to normal controls?
   b) Do PBMCs from asthmatic patients show greater Th2 or Th17 cytokine production in response to allergen in comparison to normal controls?
   c) Do PBMCs from normal controls show greater Th1 or T regulatory cytokine production in response to allergen in comparison to asthmatic patients?

2. To investigate the relationship between PBMC responses to allergen and clinical asthma symptoms:
   a) Does PBMC activation and inflammatory cytokine production correspond with atopic asthmatic patients’ known asthma triggers?
      i. Do known asthma triggers stimulate Th2 or Th17 cytokine production by a patient’s PBMCs?
      ii. Do non-trigger allergens stimulate the production of Th1 or T regulatory cytokines?

3. To investigate PBMC responses of nonatopic asthmatic patients to allergen:
   a) Do PBMCs from nonatopic asthmatic patients become activated in response to allergen?
   b) Do PBMCs from nonatopic asthmatic patients produce inflammatory cytokines in response to allergen?

4. To investigate differences in PBMC responses to allergen between different groups of asthmatic patients:
   a) Is allergen-stimulated PBMC activation able to discriminate:
      i. Atopic asthma vs nonatopic asthma
      ii. Eosinophilic bronchitis vs noneosinophilic bronchitis
   b) Is cytokine production by allergen-stimulated PBMCs able to discriminate:
      i. Atopic asthma vs nonatopic asthma
      ii. Eosinophilic bronchitis vs noneosinophilic bronchitis
      iii. Neutrophilic vs nonneutrophilic bronchitis
5. To investigate the effects of steroid treatment on allergen-induced PBMC responses. Is there a difference in response to allergen between the following treatment groups:
   a) 0-500 mg inhaled corticosteroids (ICS)
   b) 500-1000 mg ICS
   c) > 1000 mg ICS
   d) 0-10 mg Prednisone
   e) > 10 mg Prednisone

**EXPERIMENTAL METHODS**

**ETHICS**

This study was approved by the Research Ethics Board at St. Joseph's Hospital in Hamilton, Ontario.

**PATIENT CHARACTERISTICS**

The patients in this study are followed at the Firestone Institute for Respiratory Health at St. Joseph's Hospital in Hamilton, Ontario. They have been diagnosed with asthma based on a ≥ 12% improvement in FEV₁ after inhalation of salbutamol and a PC₂₀ of < 8 mg/mL. Both of these measurements demonstrate that the patients are experiencing a wide variation in airflow over a short period of time. Salbutamol is a bronchodilator and FEV₁ refers to forced expiratory volume in one second, or the volume of air that an individual can forcibly breathe out in one second. PC₂₀ refers to the provocative concentration of inhaled methacholine (a bronchoconstrictor) required to cause a 20% fall in FEV₁.

Asthmatic patients have also been sub-classified as atopic or nonatopic and eosinophilic or noneosinophilic. Atopy has been defined as a positive skin-prick test to one or more allergens. Eosinophilic asthmatic patients have been defined as having > %3 sputum eosinophils on > 2 occasions. It has also been noted if a patient has experienced a neutrophilic asthma exacerbation, defined as a sputum total cell count > 10 x 10⁶ of which ≥ 65% are neutrophils at the time of exacerbation.

Normal controls have been identified on the basis of PC₂₀ > 16 mg/mL, a negative skin-prick test, total serum IgE < 120 units/mL, and no history of asthma or allergic disease. They are not patients followed at the Firestone.

*See Appendix 1: Patient Characteristics*

**SKIN PRICK TEST – ASSESSMENT OF ATOPY**

Liquid preparations from 19 common allergens were placed on the patient’s skin which was then pricked with a lancet. Histamine was used as a positive control and saline as a negative control. After 20 minutes, the skin reactions of the patients were assessed. A wheal reaction > 3 mm was considered a positive result, indicating atopy.
Sputum Induction and Processing – Assessment of Type of Bronchitis

Samples were obtained by having the patients breathe in nebulized saline for 7 minutes and then cough up sputum. This process was repeated 3-4 times until an appropriate size sample was obtained (approximately half the size of a pea). Patients' FEV$_1$ was assessed prior to sample induction and between saline inhalations. Sputum induction was stopped if a patient's FEV$_1$ fell by 200 mL and by 20% or more from baseline, and the patient was treated with salbutamol.

For processing, sputum samples were sent to certified medical laboratory technologists who identified total and differential cell counts using the ACCUFILTER sputum processing kit.

Allergen Selection

Prior to blood collection, allergens were plated in a 96-well plate at a concentration of 200 ug/mL in AIM-V media and frozen. Since the patient cohort under investigation displays year-round asthma, we selected allergens that the patients would most likely have continuous exposure to, such as animal dander or molds that can grow indoors. All allergens were sourced from Greer Laboratories. Four microbial antigens implicated in lung infection have also been included: three surface proteins from *Streptococcus pneumoniae* and one surface protein from *Haemophilus influenzae*. These microbial proteins were kindly provided by the lab of Dr. Wayne Thomas at the University of Western Australia. Media alone was used as a negative control and the superantigen Staphylococcal enterotoxin B (SEB) was used as a positive control.

<table>
<thead>
<tr>
<th>MITES</th>
<th>INSECTS</th>
<th>EPITHELIA AND DANDER</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Mites" /></td>
<td><img src="image" alt="Insects" /></td>
<td><img src="image" alt="Epithelia and Dander" /></td>
</tr>
<tr>
<td><img src="image" alt="House Dust Mite - Dermatophagoides farinae" /></td>
<td><img src="image" alt="Cockroach, German - Blatella germanica" /></td>
<td><img src="image" alt="Cat Dander/Antigen - Felis catus (domesticus)" /></td>
</tr>
<tr>
<td><img src="image" alt="House Dust Mite - Dermatophagoides pteronyssinus" /></td>
<td><img src="image" alt="House Dust Mite - Euroglyphus maynei" /></td>
<td><img src="image" alt="Cat Epithelia - Felis catus (domesticus)" /></td>
</tr>
<tr>
<td><img src="image" alt="Food/Storage Mite - Lepidoglyphus destructor" /></td>
<td><img src="image" alt="Food/Storage Mite - Acarus siro" /></td>
<td><img src="image" alt="Dog Dander, Mixed-Breed - Canis familiaris" /></td>
</tr>
<tr>
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<td><img src="image" alt="Hamster Epithelia - Mesocricetus auratus" /></td>
<td><img src="image" alt="Mouse Epithelia - Mus musculus" /></td>
</tr>
<tr>
<td><img src="image" alt="Rat Epithelia - Rattus norvegicus" /></td>
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<td><img src="image" alt="Rat Epithelia - Rattus norvegicus" /></td>
</tr>
</tbody>
</table>
### FUNGI

<table>
<thead>
<tr>
<th>Alternaria alternaria - <em>Alternaria tenuis</em></th>
<th>Aspergillus fumigatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aureobasidium pullulans - <em>Pullularia pullans</em></td>
<td>Botrytis cinerea</td>
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<tr>
<td>Candida albicans</td>
<td>Cladosporium herbarum</td>
</tr>
<tr>
<td>Cladosporium sphaerospermum - <em>Hormodendrum hordei</em></td>
<td>Epidermophyton floccosum</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>Helminthosporium solani- <em>Spondylocladium atrovirens</em></td>
</tr>
<tr>
<td>Microsporum canis</td>
<td>Mucor circinelloides f. lusitanicus - <em>Mucor racemosus</em></td>
</tr>
<tr>
<td>Penicillium notatum</td>
<td>Phoma betae</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>Trichophyton rubrum</td>
</tr>
</tbody>
</table>

### GRASS

- Timothy - *Phleum pratense*

### WEEDS

- Mugwort, Common - *Artemisia vulgaris*
- Ragweed, Short - *Ambrosia artemisiifolia*

### MICROBIAL ANTIGENS

<table>
<thead>
<tr>
<th><em>Streptococcus pneumoniae</em> - PspA1</th>
<th><em>Streptococcus pneumoniae</em> - PspA2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pneumoniae</em> - PspC</td>
<td><em>Haemophilus influenzae</em> - Omp p6</td>
</tr>
</tbody>
</table>

## CELL CULTURE

At **DAY 0**, 30 mL of blood was collected from each patient into heparin-coated green top tubes (to prevent coagulation). Peripheral blood mononuclear cells were then separated from whole blood (T cells, monocytes, B cells, and NK cells). To isolate PBMCs, 25 mL of blood was layered onto 15 mL of Histopaque 1077 and centrifuged at 400 RCF for 30 minutes at 25°C with slow acceleration and deceleration. The buffy coat was removed and washed in 40 mL of serum-free AIM-V media at 250 RCF for 10 minutes at 25°C with maximum acceleration and deceleration. The cells were resuspended in 3 mL of AIM-V media, counted using a Countess automatic cell counter, and brought up to a final concentration of 2.5 x 10^6 cells/mL. 100 μL of cells (2.5 x 10^5 cells) were added to each well of premade plates and incubated for 5 days at 37°C.

## CYTOKINE MEASUREMENT

At **DAY 5**, 100 μL of supernatant was removed from each well for cytokine analysis by ELISA (eBioscience). 100 μL of AIM-V media was added to each well as a replacement. ELISA results are displayed in pg/mL of cytokine produced under
each condition. ELISA sensitivities are as follows: IL-5: 4-500 pg/mL, IL-17A: 4-500 pg/mL, IL-23: 15-2000 pg/mL, IL-10: 2-300 pg/mL, IFN-γ: 4-500 pg/mL.

**Proliferation Assay**

At **DAY 6**, the PBMCs were pulsed with radioactive \(^{3}\)H-thymidine (0.5 μCi per well) and incubated for 16 hours. Since thymidine is incorporated into the DNA of new cells during cell division, the amount of \(^{3}\)H-thymidine taken up by cells in culture indicates their degree of proliferation.

At **DAY 7**, the PBMCs were harvested onto glass fibre filter mats and assessed for proliferation (by way of \(^{3}\)H-thymidine incorporation) using a MicroBeta counter. The MicroBeta counter detects luminescence from the \(^{3}\)H label and expresses this in the units counts per minute (CCPM). Proliferation results are displayed as a delta measurement, which indicates the degree of PBMC proliferation seen under each condition in comparison to that seen with media alone. To generate delta measurements, proliferation under media alone has been subtracted from proliferation under each experimental stimulus. This delta measurement can be used as an index for T cell activation.

**Statistics**

Differences between patient groups were analyzed using unpaired Mann-Whitney t tests. p values < 0.05 are considered significant. Data was graphed showing the standard error of the mean (SEM) and statistical significance (*).

**Results**

All consolidated patient results are presented in Appendix I and all individual patient results are presented in Appendix III. Both PBMC proliferation (expressed as a delta measurement) and cytokine data are shown for each individual antigen as well as for each antigen class: Mites and Insects, Animal Dander and Epithelia, Fungi, Microbial Proteins, and Grass and Weeds.

**Objective 1: Investigating Differences in PBMC Responses to Allergen Between Asthmatic Patients and Normal Controls**

a) PBMCs from asthmatic patients did not display higher proliferation in response to allergen when compared with normal controls. This suggests that there is a comparable level of T cell activation in response to these antigens between the two groups.

*See Figures 1.1 and 1.2.*
Figure 1.1 Delta: Asthma vs Normal

Asthma (n = 39)  
Normal Controls (n = 8)

Figure 1.2 Delta Grouped: Asthma vs Normal

Asthma (n = 39)  
Normal Controls (n = 8)
b) PBMCs cells from asthmatic patients did not display significantly greater levels of Th2 or Th17 cytokine production in response to allergen. Although it appears that asthmatic patients produce more IL-5 in response to allergen than normal controls, this trend did not reach statistical significance. 

See Figures 2.1 and 2.2 for IL-5 data
See Figures 3.1 and 3.2 for IL-17A data.
See Figures 4.1 and 4.2 for IL-23 data.

**Figure 2.1 IL-5: Asthma vs Normal**
Figure 2.2 IL-5 Grouped: Asthma vs Normal

Figure 3.1 IL-17A: Asthma vs Normal
Figure 3.2 IL-17A Grouped: Asthma vs Normal

Figure 4.1 IL-23: Asthma vs Normal
PBMCs cells from normal controls did not display greater levels of Th1 or T regulatory cytokine production in response to allergen. The difference between the IL-10 responses of asthmatic patients and normal controls to the “Grass and Weeds” antigen class was statistically significant. This was due to the response to mugwort, and was not representative of either ragweed or timothy grass, or any of the other allergens on the panel. 

*See Figures 5.1 and 5.2 for IL-10 data.*

There was a trend for higher IFN-γ responses in the normal controls that reaches statistical significance only in the “Grass and Weeds” antigen class, again due to the response to mugwort. 

*See Figures 6.1 and 6.2 for IFN-γ data.*
Figure 5.1 IL-10: Asthma vs Normal

Figure 5.2 IL-10 Grouped: Asthma vs Normal
Figure 6.1 IFN-gamma: Asthma vs Normal

Figure 6.2 IFN-gamma Grouped: Asthma vs Normal
OBJECTIVE 2: Investigating The Relationship Between PBMC Responses to Allergen and Clinical Asthma Symptoms

a) PBMC proliferation and inflammatory cytokine production did not necessarily correspond with known asthma triggers in atopic asthmatic patients. Atopic patients produced cytokines to a variety of allergens, some of which are known to trigger their asthma, and some of which are not. They also failed to produce cytokines in response to some of their known asthma triggers.

As an example, we can consider atopic asthmatic patient 7, who is known to be sensitive to dust mite. This patient showed dominant IL-5 and IL-17A production to dust mite, but also to cat dander, mouse epithelium, and grass. See Figures 11.11 and 11.12 for this patient’s cytokine data.

Another interesting example is atopic asthmatic patient 10, who is sensitive to the mold *Alternaria alternaria*, cat, horse, feathers, and grass. This patient showed high IL-5 in response to cat epithelia and cat dander, but no cytokine production to *Alternaria* or grass.

See Figure 11.17 and 11.18 for this patient’s cytokine data.

See Figures 11.1 – 11.32 for all individual atopic asthmatic patients’ cytokine data.

![Figure 11.11 Atopic Asthmatic Patient 7 - Eosinophilic Allergies: dust mite](image-url)
Figure 11.12 Atopic Asthmatic Patient 7 - Eosinophilic Allergies: dust mite

Stimulus

Figure 11.17 Atopic Asthmatic Patient 10 - Eosinophilic Allergies: Alternaria, cat, horse, feathers, grass
OBJECTIVE 3: PBMC Responses of Nonatopic Asthmatic Patients to Allergen

a) Nonatopic asthmatic patients did not display increased PBMC proliferation in response to allergen when compared to normal controls. See Figure 1.7.
b) PBMCs from nonatopic asthmatic patients did produce inflammatory cytokines in response to allergen, although cytokine levels were not statistically different from those produced by normal controls. Although it appears that nonatopic asthmatics produced more IL-5 in response to allergen than normal controls, this trend did not reach statistical significance. 

*See Figure 2.9 for IL-5 data.*

*See Figure 3.9 for IL-17A data.*

There was a trend for more IL-23 production in normal controls than in nonatopic asthmatics. 

*See Figure 4.9 for IL-23 data.*

**Figure 2.9 IL-5 Grouped: Nonatopic Asthma vs Normal**
Figure 3.9 IL-17A Grouped: Nonatopic Asthma vs Normal

![Graph showing IL-17A levels for Nonatopic Asthma and Normal Controls.]

Figure 4.9 IL-23 Grouped: Nonatopic Asthma vs Normal

![Graph showing IL-23 levels for Nonatopic Asthma and Normal Controls.]

* p = 0.0260
OBJECTIVE 4: Differences in PBMC Responses to Allergen Between Different Groups of Asthmatic Patients

a) Allergen-stimulated PBMC proliferation was unable to distinguish different patient groups.
   i. No difference in PBMC proliferation was seen between atopic and nonatopic patient groups. 
      See Figures 1.3 and 1.4.
   ii. No difference in PBMC proliferation was seen between eosinophilic and noneosinophilic patient groups. 
      See Figures 1.5 and 1.6.

**Figure 1.3 Delta: Nonatopic vs Atopic Asthma**
Figure 1.4 Delta Grouped: Nonatopic vs Atopic Asthma

Figure 1.5 Delta: Eosinophilic vs Noneosinophilic
b) Cytokine production by allergen-stimulated PBMCs was unable to definitively distinguish different patient groups.
   i. Atopic asthma vs nonatopic asthma. Although there were trends for higher IL-5, IL-23, and IFN-γ production by atopic asthmatic patients, these were not statistically significant.
   See Figures 2.3 and 2.4 for IL-5 data.
   See Figures 3.3 and 3.4 for IL-17A data.
   See Figures 4.3 and 4.4 for IL-23 data.
   See Figures 5.3 and 5.4 for IL-10 data.
   See Figures 6.3 and 6.4 for IFN-γ data.
Figure 2.3 IL-5: Atopic vs Nonatopic Asthma

Figure 2.4 IL-5 Grouped: Atopic vs Nonatopic Asthma
Figure 3.3 IL-17A: Atopic vs Nonatopic Asthma

Figure 3.4 IL-17A Grouped: Atopic vs Nonatopic Asthma
Figure 4.3 IL-23: Atopic vs Nonatopic Asthma

Figure 4.4 IL-23 Grouped: Atopic vs Nonatopic Asthma
Figure 5.3 IL-10: Atopic vs Nonatopic Asthma

![Figure 5.3 IL-10: Atopic vs Nonatopic Asthma](image)

Figure 5.4 IL-10 Grouped: Atopic vs Nonatopic Asthma

![Figure 5.4 IL-10 Grouped: Atopic vs Nonatopic Asthma](image)
Figure 6.3 IFN-gamma: Atopic vs Nonatopic Asthma

Figure 6.4 IFN-gamma Grouped: Atopic vs Nonatopic Asthma
Eosinophilic bronchitis vs noneosinophilic bronchitis. There were trends for increased IL-5, IL-17A, and IFN-γ production by patients with noneosinophilic bronchitis. Interestingly, the noneosinophilic group produced significantly more IL-5 in response to the “Mites and Insects” and “Animal Dander and Epithelia” allergen classes. This group also produced significantly more IL-17A to the “Animal Dander and Epithelia” class.

See Figures 2.5 and 2.6 for IL-5 data.
See Figures 3.5 and 3.6 for IL-17A data.
See Figures 4.5 and 4.6 for IL-23 data.
See Figures 5.5 and 5.6 for IL-10 data.
See Figures 6.5 and 6.6 for IFN-γ data.

**Figure 2.5 IL-5: Eosinophilic vs Noneosinophilic**

![Graph showing IL-5 levels for Eosinophilic and Noneosinophilic groups](image-url)
Figure 2.6 IL-5 Grouped: Eosinophilic vs Noneosinophilic

Stimulus

Figure 3.5 IL-17A: Eosinophilic vs Noneosinophilic

Stimulus
Figure 3.6 IL-17A Grouped: Eosinophilic vs Noneosinophilic

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Stimulus

0
50
100
150
200
250

Eosinophilic (n = 32)
Noneosinophilic (n = 10)

*p = 0.0116

Figure 4.5 IL-23: Eosinophilic vs Noneosinophilic

FSDM L dest
FSDM A siro
HDM D pteron
HDM D farinae
HDM E maynei
C Roach Peri Am
C Roach B Ger
Cap Ep F catus
Cat Dand
Dog Dand
Rat Ep
Ham Ep
Mouse Ep
Alternaria
A fum
A pull
B cinerea
B chondr
C herba
C sphaer
E floc
F solani
H solani
M canis
M racem
P betae
P notatum
Psp A1
Psp A2
Psp C
Omp p6
Timothy
Mugwort
Ragweed

Stimulus

0
100
200
300
400
500

Eosinophilic (n = 32)
Noneosinophilic (n = 10)
Figure 4.6 IL-23 Grouped: Eosinophilic vs Noneosinophilic

Figure 5.5 IL-10: Eosinophilic vs Noneosinophilic
Figure 5.6 IL-10 Grouped: Eosinophilic vs Noneosinophilic

Figure 6.5 IFN-gamma: Eosinophilic vs Noneosinophilic
iii. Neutrophilic vs nonneutrophilic bronchitis. No significant differences were seen between these two groups. See Figures 2.7 and 2.8 for IL-5 data. See Figures 3.7 and 3.8 for IL-17A data. See Figures 4.7 and 4.8 for IL-23 data. See Figures 5.7 and 5.8 for IL-10 data. See Figures 6.7 and 6.8 for IFN-γ data.
Figure 2.7 IL-5: Neutrophilic vs Nonneutrophilic

Figure 2.8 IL-5 Grouped: Neutrophilic vs Nonneutrophilic
Figure 3.7 IL-17A: Neutrophilic vs Nonneutrophilic

Stimulus

Figure 3.8 IL-17A Grouped: Neutrophilic vs Nonneutrophilic

Stimulus

Nonneutrophilic (n = 36)
Neutrophilic (n = 6)
Figure 4.7 IL-23: Neutrophilic vs Nonneutrophilic

Figure 4.8 IL-23 Grouped: Neutrophilic vs Nonneutrophilic
Figure 5.7 IL-10: Neutrophilic vs Nonneutrophilic

Nonneutrophilic (n = 35)
Neutrophilic (n = 6)

Figure 5.8 IL-10 Grouped: Neutrophilic vs Nonneutrophilic

Nonneutrophilic (n = 35)
Neutrophilic (n = 6)
Figure 6.7 IFN-gamma: Neutrophilic vs Nonneutrophilic

Figure 6.8 IFN-gamma Grouped: Neutrophilic vs Nonneutrophilic
**OBJECTIVE 5: Effects of Steroids on PBMC Responses to Allergen**

Average IL-5 production declined significantly with increasing steroid dosage. This effect was not seen with PBMC proliferation or production of other cytokines.

*See Figures 9.2 and 9.7 for IL-5 data.*

*See Figure 9.1 for PBMC proliferation data.*

*See Figures 9.3-9.6 for IL-17A, IL-23, IL-10 and IFN-γ data.*
**DISCUSSION**

**PBMC Proliferation**

PBMC proliferation in response to allergen did not differ between any of the patient groups in this study: asthma vs normal, atopic vs nonatopic asthma, or eosinophilic bronchitis vs noneosinophilic bronchitis. This suggests that allergen-induced PBMC proliferation, and possibly T cell activation, in peripheral blood is not a good marker of clinical disease in asthma. It cannot distinguish between the presence or absence of disease, or between different phenotypes of asthma.

**Cytokines**

The results from this project indicate that peripherally produced cytokines, or at least the cytokines selected for this study, do not act as effective, specific biomarkers that can distinguish 1) asthma vs normal controls 2) atopic asthma vs nonatopic asthma 3) eosinophilic bronchitis vs noneosinophilic bronchitis or neutrophilic bronchitis vs nonneutrophilic bronchitis. All patient groupings in this study exhibited fairly similar patterns of cytokine production, regardless of phenotype. See Figures 7.1-7.8. This observation was not altered when we focused in on allergens deemed to be especially clinically relevant – those featured in the common skin-prick test. See Figures 8.1-8.8.

In some cases, wide patient variability, indicated by large SEM values, contributed to a lack of statistical significance of the cytokine data. Though this variability in cytokine production makes it difficult to draw solid conclusions based on statistics, it does support the idea of asthma as a heterogeneous disease in which individual patients’ asthmatic responses to stimuli are highly varied. Such variability in cytokine responses has been documented in other studies. Mentioned previously in this report, Mori’s group found IL-5 production in response to *Candia*...
*albicans* from nonatopic asthmatic patients ranged from less than 50 pg/mL to greater than 250 pg/mL\(^5\).

**Asthma vs Normal**

We predicted that asthmatic patients would display higher levels of Th2 and Th17 associated cytokines in response to allergen, and that normal controls would display higher levels of Th1 or T regulatory associated cytokines. This prediction was not confirmed by the data.

If we look at the consolidated patient data as a whole, no statistically significant differences in cytokine production between the two groups exist and no clear trends emerge.

If we examine each patient individually, the response to allergen by normal controls is dominated by IL-10 and IFN-\(\gamma\) production. For example, if we consider normal control 14 (*see Figures 12.13 and 12.14*): this individual produces IL-5, IL-17A, and IL-23 in response to allergen, but the dominant cytokines expressed in response to each class of allergens are IL-10 and IFN-\(\gamma\). This finding makes it tempting to conclude that the expression of Th2 and Th17 cytokines may not lead to asthma or other allergic disease in a person if they are “balanced” by the expression of Th1 and T regulatory cytokines. However, if we examine individual asthmatic patients, the same trend in cytokine expression can be observed. For example, the cytokine response to allergen is dominated by IL-10 and IFN-\(\gamma\) in nonatopic asthmatic patient 7 (*see Figures 10.13 and 10.14*) and atopic asthmatic patient 17 (*see Figures 11.31 and 11.32*), among others. Not all asthmatic patients follow this trend, as is seen in nonatopic asthmatic patient 13 (*see Figures 10.25 and 10.26*), who displays predominantly high IL-17A levels. Overall, the main message illustrated by this comparison is that we cannot look at a patient’s cytokine data and determine whether that patient has asthma or is a normal control. The amount of cytokine and the balance of cytokine types produced in response to allergen is not an effective way to distinguish disease from non-disease in asthma.
Figure 10.13 Nonatopic Asthmatic Patient 7 - Eosinophilic

Figure 10.14 Nonatopic Asthmatic Patient 7 - Eosinophilic
**Figure 11.31** Atopic Asthmatic Patient 17 - Eosinophilic Allergies: cat, horse, feathers, dust mite

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

**Figure 11.32** Atopic Asthmatic Patient 17 - Eosinophilic Allergies: cat, horse, feathers, dust mite

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma
Figure 10.25 Nonatopic Asthmatic Patient 13 - Neutrophilic

Figure 10.26 Nonatopic Asthmatic Patient 13 - Neutrophilic

Atopic and Nonatopic Asthma
We expected cytokine levels to be higher in atopic asthmatic patients than nonatopic asthmatic patients. Atopic asthmatic patients have an IgE-mediated mechanism of hypersensitivity at work in addition to a T cell-mediated mechanism, while our prediction was that nonatopic asthmatics only displayed the T cell-mediated mechanism. Therefore, we predicted that the combined effect of both pathways would lead to greater levels of cytokine production. No significant differences were found between the groups for any of the five cytokines, despite a trend in higher amounts of IL-5 in atopic asthmatics.

We anticipated that atopic asthmatic patients would produce pro-inflammatory cytokines in response to asthma triggers they have known sensitivities to. We also expected a response to non-trigger allergens characterized by the absence of Th2 and/or Th17 inflammation or balanced by a Th1 and/or T regulatory response. Instead, atopic asthmatic patients produced pro-inflammatory cytokines to a number of different allergens on our allergen panel, and this cytokine profile did not necessarily match their known sensitivities.

The results from atopic asthmatic patients indicate that pro-inflammatory cytokine production, at least by PBMCs, does not necessarily follow exposure to allergens a person is sensitized to. Individuals may exhibit inflammatory cytokine responses to allergens that do not cause them to have any clinical symptoms of asthma or allergic disease. They may also lack inflammatory cytokine responses to allergens they have known sensitivities to. In addition, IL-5, IL-17A and IL-23 responses are not consistent across our allergen panel for each patient.

Cytokine profile clearly does not reflect sensitivity profile. From this, we can conclude that the skin-prick test is not necessarily indicative of a patient’s cellular response, as measured by inflammatory cytokine production by PBMCs, to aeroallergen. This leads to the question: what is important in driving asthmatic response - IgE or inflammatory cytokines? Or are both required for a meaningful allergic or asthmatic response to occur? If this is true, local IgE production in the lung may play an important role in asthma pathogenesis. Also, despite their strong association with asthma, the cytokines investigated in this project may not be integral to driving disease in asthma patients.

We predicted that nonatopic asthmatic patients would display distinct pro-inflammatory cytokine responses to some allergens on our panel. This could indicate a previously undetected T cell-mediated sensitivity to allergen in these patients that could be driving their asthma. If this was observed, we planned to challenge nonatopic patients with allergens they showed strong pro-inflammatory cytokine responses to and look for a late-phase asthmatic response. Our work did show that nonatopic asthmatic patients produce IL-5, IL-17A, and IL-23 to various allergens. However, the data from the atopic asthmatic group showed that pro-inflammatory cytokine production is not indicative of sensitivity to allergen. Therefore, it is difficult to make any predictions about clinically relevant sensitivity to allergen in our nonatopic asthmatic group based on their cytokine responses.

**Type of Bronchitis**

We expected to see greater IL-5 responses in asthma patients with a history of eosinophilic bronchitis, as well as greater IL-17A and/or IL-23 responses in
patients with a history of neutrophilic bronchitis. Neither of these predictions was supported by the data.

The most difficult data set to explain is IL-5 production from eosinophilic vs noneosinophilic asthma patients. In two categories of allergic stimuli, “Mites and Insects” and “Animal Dander and Epithelia”, noneosinophilic patients produced statistically significant higher quantities of IL-5. This was an unexpected result, since it has been shown that levels of IL-5 mRNA+ cells in the airway correlate with the total number of infiltrating eosinophils and activated eosinophils and that the administration of anti-IL-5 decreases eosinophil recruitment to the lung and subsequently improves asthma. Our results seem to be in contradiction with these findings, indicating that peripheral blood IL-5 responses to allergen may not pay a clinically relevant role in airway eosinophilia. It is possible that a significantly stronger IL-5 response to allergens is occurring locally in the lungs of eosinophilic asthmatics, and we are just unable to detect this in the periphery. Alternatively, this discrepancy may be due to differences in steroid use between the two groups. The patients in our study with eosinophilic bronchitis are taking higher doses of corticosteroids, which have been shown to suppress IL-5 production by PBMCs. It is possible that in the absence of this steroid treatment, eosinophilic asthmatics would produce significantly higher levels of IL-5 in response to allergens than noneosinophilic asthmatics.

Though not statistically significant, our data reveals an elevated IL-17A response to microbial antigens by asthmatic patients with a history of neutrophilic asthma exacerbations, which may have interesting implications for the idea of “neutrophilic asthma”. It could indicate that neutrophilic asthma is not in fact a distinct phenotype seen in patients with more severe disease, but is actually the product of a strong IL-17A (neutrophil-recruiting) recall response to microbial antigens in certain individuals. Therefore, exposure to exogenous microbes is still the trigger for this strong neutrophilic response. Based on our limited data, we should be cautious about how much emphasis is placed on this observation. Nonetheless, it would be interesting to investigate whether this elevated IL-17A response to microbial antigens in the blood is also seen locally in the lung.

If we consider individual patients, it is clear that allergen-induced PBMC cytokine profiles do not necessarily match what we would expect based on known clinical characteristics. In some patients, such as nonatopic patient 13 (see Figure 10.26), cytokine responses to allergen match what we would predict based on phenotype. This patient has a history of neutrophilic asthma exacerbations and has responses to allergens characterized by dominant IL-17A production. However, nonatopic patient 8 is a noneosinophilic patient who displays strong IL-5 responses (see Figure 10.16). As another example, we can consider atopic asthmatic patient 5 (see Figure 11.12) who is known to be sensitive to dust mite and mold and has a history of airway eosinophilia. The patient’s cytokine responses to the “Mites and Insects” and “Fungi” classes of allergen are dominated by IL-17A and IFN-γ production, as opposed to IL-5. Again, the message here is that we cannot look at a patient’s PBMC cytokine responses to allergen and predict their phenotype.
Figure 10.26 Nonatopic Asthmatic Patient 13 - Neutrophilic

![Graph showing cytokine production](image)

- **Mites/Insects**
- **Animal Dander/Epithelia**
- **Fungi**
- **Microbial**
- **Grass/Weeds**

Stimulus

0
100
200
300
400
IL-5
IL-17A
IL-23
IL-10
IFN-gamma

pg/mL

Figure 10.16 Nonatopic Asthmatic Patient 8 - Noneosinophilic

![Graph showing cytokine production](image)

- **Mites/Insects**
- **Animal Dander/Epithelia**
- **Fungi**
- **Microbial**
- **Grass/Weeds**

Stimulus

0
200
400
600
800
IL-5
IL-17A
IL-23
IL-10
IFN-gamma

pg/mL
Steroid Dosage

It is important to acknowledge the effect that steroid treatment may have had on the outcomes of this study, as our results show a significant decrease in IL-5 production with increasing steroid dosage. Had the eosinophilic patients in our study not been on high doses of steroids, a more significant role for PBMC-produced IL-5 as a biomarker of their disease may have emerged.

CONCLUDING REMARKS

The main findings of this project are as follows:

1. Antigen-induced PBMC proliferation does not distinguish asthma from normal controls, atopic asthma from nonatopic asthma, or differentiate between different types of bronchitis.
2. The cytokines IL-5, IL-17A, IL-23, IL-10, and IFN-γ produced by PBMCs in response to allergen are not good biomarkers of disease in asthma.
   a. They do not distinguish asthma from normal controls, atopic asthma from nonatopic asthma, or differentiate between different types of bronchitis.
3. Pro-inflammatory cytokine production by PBMCs does not necessarily match sensitization profile. The skin prick test, indicative of IgE production, does not always predict a patient’s cellular response to allergen.
The cytokine data from this project reveals an apparent disconnect between PBMC activation/pro-inflammatory cytokine production and clinically relevant disease. There are two ways in which our findings can be interpreted.

The first is to assume that responses to allergen measured in patients’ peripheral blood are representative of the cellular activity in their airways. Several studies mentioned previously in this report have suggested that cytokines present in serum or produced by peripheral blood cells accurately represent the environment of the airways and can act as biomarkers of lung disease. In this case, we would have to conclude that the cytokines investigated in this study (as produced by T cells, macrophages, dendritic cells, B cells, or NK cells) do not play a significant role in driving disease in asthma and cannot be used as biomarkers for asthmatic phenotypes. This conclusion still leaves room for significant cytokine production from other sources, such as mast cell production of IL-5, but calls into question a central role for the T cell in asthma.

However, it is important to recognize the limitations of using peripheral blood cells to study a disease of the lungs. Therefore, the second interpretation of our data is that allergen-induced peripheral blood mononuclear cell responses to allergen are not good biomarkers of disease in asthma. PBMC activity may or may not be representative of what is actually going on in patient airways, but it does not appear to drive disease in asthma. No differences in PBMC activity were seen in patients with asthma, compared with normal controls, or between patients with different asthmatic phenotypes. It is not possible to determine a patient’s disease state, atopic status, or type of bronchitis by looking at their PBMC responses to allergen. We believe this is the most correct conclusion to draw from the results of our study. This opens the door for an interesting follow-up project: investigating the cellular responses to allergen by samples taken from our patients’ airways and compare these with their PBMC data. This would determine whether or not PBMC responses to allergen of asthma patients are representative of their airway environment and could clarify the role of T cell cytokines in driving asthma.

This project has highlighted both the importance and the uniqueness of the local environment in patients with asthma. Factors driving asthma may be unique to the airways and not manifest systemically in a patient. For example, this suggests a central role for IL-5 production by mast cells and T cells in the lung, potentially mediated by local IgE production, in patients with airway eosinophilia. We may not be able to detect this IL-5 and IgE production using PBMC assays or the skin-prick test, but it certainly holds clinical significance for the patient. Future studies may shed light on the ability of locally produced T cell cytokines to act as biomarkers in asthma, but our data indicates that these cytokines produced in the periphery are unable act as such.
## Appendix I: Patient Characteristics

<table>
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<th>Age</th>
<th>Gender</th>
<th>Asthma Status</th>
<th>Atopic Status</th>
<th>Allergies</th>
<th>Sputum Eos</th>
<th>Sputum Neuts</th>
<th>FEV1</th>
<th>FEV1 %</th>
<th>FEV1/VC</th>
<th>&lt; 500 mg ICS</th>
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<td>Yes</td>
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M.Sc. Thesis – E. Simms; McMaster University – Medical Sciences (II)
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<td>Grass, peanuts, shellfish, animals</td>
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<td>M</td>
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<td>Dust mite</td>
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<td>No</td>
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</tr>
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<td>f1000</td>
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<td>ES013AA-E</td>
<td>74</td>
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<td>Yes</td>
<td>Cat, dog, horse</td>
<td>Yes</td>
<td>No</td>
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<td>ES014AA-E</td>
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<td>p2400 &lt;s800&gt; 5 alt. days</td>
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<td>ES015AA-E</td>
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<td>D. far, D. pter, grass, ragweed</td>
<td>Yes</td>
<td>No</td>
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<td>79</td>
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M.Sc. Thesis – E. Simms; McMaster University – Medical Sciences (II)
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<th>Alcohol</th>
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APPENDIX II: CONSOLIDATED PATIENT RESULTS

Contains:
1. PBMC Proliferation Data
2. Interleukin-5 Data
3. Interleukin-17A Data
4. Interleukin-23 Data
5. Interleukin-10 Data
6. Interferon-γ Data
7. All Cytokines
8. All Cytokines: Skin Prick Test
9. Steroid Dosage Data
Figure 1.1 Delta: Asthma vs Normal

Stimulus

- FSDM L dest
- FSDM A siro
- HDM D pteron
- HDM D farinae
- HDM E maynei
- C Roach Peri Am
- C Roach B Ger
- Cap Ep F catus
- Cat Ep F catus
- Dog Ep B Ger
- Rat Ep B Ger
- Ham Ep B Ger
- Mouse Ep B Ger
- Alternaria
- A fum
- A pull
- B cinerea
- C herb
- C sphaer
- E floc
- F solani
- H solani
- M canis
- M race
- P notatum
- P betae
- S cervis
- T rubrum
- Psp A1
- Psp A2
- Psp C
- Omp p6
- Timothy
- Ragweed

Asthma (n = 39)
Normal Controls (n = 8)
Figure 1.2 Delta Grouped: Asthma vs Normal

Stimulus

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

CCPM

Asthma (n = 39)
Normal Controls (n = 8)
Figure 1.3 Delta: Nonatopic vs Atopic Asthma

- FSDM L dest
- FSDM A siro
- HDM D pteron
- HDM D farinae
- HDM E maynei
- C Roach Peri Am
- C Roach B Ger
- Cap Ep F catus
- Cat Ep Dand
- Dog Ep Dand
- Rat Ep Dand
- Ham Ep Dand
- Mouse Ep Dand
- Alternaria
- A fum
- A pul
- B cinerea
- C sphaer
- C herb
- C solani
- M canis
- M race
- P notatum
- P betae
- S cervis
- T rubrum
- Psp A1
- Psp A2
- Psp C
- Omp p6
- Timothy
- Mugwort
- Ragweed

Nonatopic Asthma (n = 23)
Atopic Asthma (n = 16)
Figure 1.4 Delta Grouped: Nonatopic vs Atopic Asthma

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Nonatopic Asthma (n = 23)
Atopic Asthma (n = 16)
Figure 1.5 Delta: Eosinophilic vs Noneosinophilic

- Eosinophilic (n = 29)
- Noneosinophilic (n = 10)

Stimulus:
- CCPM
- Stimuli listed in the figure legend.
Figure 1.6 Delta Grouped: Eosinophilic vs Noneosinophilic Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

CCPM

Stimulus

Eosinophilic (n = 28)
Noneosinophilic (n = 10)
Figure 1.7 Delta Grouped: Nonatopic Asthma vs Normal

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

CCPM

Nonatopic Asthma (n = 23)
Normal Controls (n = 8)
Figure 2.1 IL-5: Asthma vs Normal

- FSDM L dest
- FSDM A siro
- HDM D pteron
- HDM D farinae
- HDM E maynei
- C Roach Peri Am
- C Roach B Ger
- Cap Ep F catus
- Cat Ep A ger
- Dog Dand
- Rat Ep
- Ham Ep
- Mouse Ep
- Alternaria
- A tum
- A pull
- B cinerea
- Candida
- C phaffi
- C sphaler
e
- E floc
- F solani
- H solani
- M canis
- M race
- P notatum
- P betae
- S cervis
- T rubrum
- Psp A1
- Psp A2
- Psp C
- Omp p6
- Timothy
- Mugwort
- Ragweed

Stimulus

Asthma (n = 42)
Normal Controls (n = 8)
Figure 2.2 IL-5 Grouped: Asthma vs Normal

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

pg/mL

- Asthma (n = 42)
- Normal Controls (n = 8)
Figure 2.3 IL-5: Atopic vs Nonatopic Asthma

![Graph showing IL-5 levels in Atopic vs Nonatopic Asthma](image)

- **Nonatopic Asthma (n = 26)**
- **Atopic Asthma (n = 16)**

**Stimulus**

- Cat Dand
- Dog Dand
- Rat Ep
- Ham Ep
- Mouse Ep
- Alternaria
- A fum
- A pull
- B cinerea
- Caudi
- C sphaer
- E floc
- F solani
- H solani
- M canis
- M race
- P notatum
- P betae
- S cervis
- Trbrum
- Psp A1
- Psp A2
- Psp C
- Omp p6
- Timothy
- Mugwort
- Ragweed

M.Sc. Thesis – E. Simms; McMaster University – Medical Sciences (II)
Figure 2.4 IL-5 Grouped: Atopic vs Nonatopic Asthma

- **Nonatopic Asthma (n = 26)**
- **Atopic Asthma (n = 16)**

**Stimulus**
- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

**pg/mL**
- 0
- 20
- 40
- 60
- 80
- 100
Figure 2.5 IL-5: Eosinophilic vs Noneosinophilic

- Eosinophilic (n = 32)
- Noneosinophilic (n = 10)

Stimulus (pg/mL):
- FSDM L dest
- FSDM A siro
- HDM D pteron
- HDM D farinae
- HDM E maynei
- C Roach Peri Am
- C Roach B Ger
- Cat Ep F catus
- Cat Dand
- Dog Dand
- Rat Ep
- Ham Ep
- Mouse Ep
- Alternaria
- A fum
- A pull
- B cinerea
- Candida
- C herba
- C sphaer
- E floc
- F solani
- H solani
- M canis
- M race
- P notatum
- P betae
- S cerevis
- T rubrum
- Psp A1
- Psp A2
- Psp C
- Omp p6
- Timothy
- Mugwort
- Ragweed
Figure 2.6 IL-5 Grouped: Eosinophilic vs Noneosinophilic

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

pg/mL

Eosinophilic (n = 32)
Noneosinophilic (n = 10)

p = 0.0374
p = 0.004
Figure 2.7 IL-5: Neutrophilic vs Nonneutrophilic

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Nonneutrophilic (n = 36)</th>
<th>Neutrophilic (n = 6)</th>
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</thead>
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<td>FSDM L dest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSDM A siro</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDM D pteron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDM D farinae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C Roach Perl Am</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C Roach B Ger</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cap Ep F catus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat Dand</td>
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<td>Dog Dand</td>
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<td>Mouse Ep</td>
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</tr>
<tr>
<td>A fum</td>
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<td>Candida</td>
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<tr>
<td>C herba</td>
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<tr>
<td>C sphaer</td>
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<td></td>
</tr>
<tr>
<td>E floc</td>
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</tr>
<tr>
<td>F solani</td>
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</tr>
<tr>
<td>H solani</td>
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<td>M caris</td>
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</tr>
<tr>
<td>M race</td>
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<td></td>
</tr>
<tr>
<td>P notatum</td>
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<td></td>
</tr>
<tr>
<td>P beta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S cervis</td>
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<tr>
<td>T rubrum</td>
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<td></td>
</tr>
<tr>
<td>Psp A1</td>
<td></td>
<td></td>
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<tr>
<td>Omp p6</td>
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<td>Timothy</td>
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<td>Mugwort</td>
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<td></td>
</tr>
<tr>
<td>Ragweed</td>
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</tbody>
</table>

Stimulus

pg/mL

Nonneutrophilic (n = 36)

Neutrophilic (n = 6)
Figure 2.8 IL-5 Grouped: Neutrophilic vs Nonneutrophilic

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

pg/mL

Nonneutrophilic (n = 36)
Neutrophilic (n = 6)
Figure 2.9 IL-5 Grouped: Nonatopic Asthma vs Normal

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Stimulus

Nonatopic Asthma (n = 26)
Normal Controls (n = 8)
Figure 3.1 IL-17A: Asthma vs Normal

Stimulus

- Asthma (n = 42)
- Normal Controls (n = 8)
Figure 3.2 IL-17A Grouped: Asthma vs Normal

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

- Asthma (n = 42)
- Normal Controls (n = 8)

pg/mL
Figure 3.3 IL-17A: Atopic vs Nonatopic Asthma

Stimulus

Nonatopic Asthma (n = 26)
Atopic Asthma (n = 16)
Figure 3.4 IL-17A Grouped: Atopic vs Nonatopic Asthma

Nonatopic Asthma (n = 26)
Atopic Asthma (n = 16)
Figure 3.5 IL-17A: Eosinophilic vs Noneosinophilic

- Eosinophilic (n = 32)
- Noneosinophilic (n = 10)

Stimulus: various allergens and fungi
Figure 3.6 IL-17A Grouped: Eosinophilic vs Noneosinophilic

- **Stimulus**
  - Mites/Insects
  - Animal Dander/Epithelia
  - Fungi
  - Microbial
  - Grass/Weeds

- **pg/mL**
  - 0
  - 50
  - 100
  - 150
  - 200
  - 250

- **Eosinophilic (n = 32)**
- **Noneosinophilic (n = 10)**

- *p = 0.0116*
Figure 3.7 IL-17A: Neutrophilic vs Nonneutrophilic

Stimulus

Nonneutrophilic (n = 36)
Neutrophilic (n = 6)
Figure 3.8 IL-17A Grouped: Neutrophilic vs Nonneutrophilic

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

pg/mL

Nonneutrophilic (n = 36)
Neutrophilic (n = 6)
Figure 3.9 IL-17A Grouped: Nonatopic Asthma vs Normal

- Nonatopic Asthma (n = 26)
- Normal Controls (n = 8)

Stimulus (pg/mL):
- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

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Figure 4.1 IL-23: Asthma vs Normal

- Asthma (n = 42)
- Normal Controls (n = 7)

Stimulus

pg/mL
Figure 4.2 IL-23 Grouped: Asthma vs Normal

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Stimulus

pg/mL

Asthma (n = 42)
Normal Controls (n = 7)

*p = 0.0230
Figure 4.3 IL-23: Atopic vs Nonatopic Asthma

Nonatopic Asthma (n = 26)
Atopic Asthma (n = 16)

Stimulus

M.Sc. Thesis – E. Simms; McMaster University – Medical Sciences (II)
Figure 4.4 IL-23 Grouped: Atopic vs Nonatopic Asthma

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

Nonatopic Asthma (n = 26)
Atopic Asthma (n = 16)
Figure 4.5 IL-23: Eosinophilic vs Noneosinophilic

- Eosinophilic (n = 32)
- Noneosinophilic (n = 10)

Stimulus

pg/mL
Figure 4.6 IL-23 Grouped: Eosinophilic vs Noneosinophilic

- **Eosinophilic (n = 32)**
- **Noneosinophilic (n = 10)**

**Stimulus**: pg/mL

- **Mites/Insects**
- **Animal Dander/Epithelia**
- **Fungi**
- **Microbial**
- **Grass/Weeds**

*p = 0.0258*
Figure 4.7 IL-23: Neutrophilic vs Nonneutrophilic

Nonneutrophilic (n = 36)
Neutrophilic (n = 6)

Stimulus
pg/mL

M.Sc. Thesis – E. Simms; McMaster University – Medical Sciences (II)
Figure 4.8 IL-23 Grouped: Neutrophilic vs Nonneutrophilic

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Stimulus

pg/mL
Figure 4.9 IL-23 Grouped: Nonatopic Asthma vs Normal

- Mites/Insects
- Animal Dander/Epitheilia
- Fungi
- Microbial
- Grass/Weeds

Nonatopic Asthma (n = 26)
Normal Controls (n = 7)

* p = 0.0260
Figure 5.1 IL-10: Asthma vs Normal

Stimulus

pg/mL

Asthma (n = 41)
Normal Controls (n = 8)
Figure 5.2 IL-10 Grouped: Asthma vs Normal

Stimulus

pg/mL

Asthma (n = 41)  Normal Controls (n = 8)

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

\( p = 0.0212 \)
Figure 5.3 IL-10: Atopic vs Nonatopic Asthma

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<tr>
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</tr>
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<td>HDM D farinae</td>
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</tr>
<tr>
<td>HDM E maynei</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C Roach Peri Am</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C Roach B Ger</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cap Ep F catus</td>
<td></td>
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</tr>
<tr>
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<tr>
<td>Cat Dand</td>
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<tr>
<td>Rat Ep</td>
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<tr>
<td>Ham Ep</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>A fum</td>
<td></td>
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</tr>
<tr>
<td>A pull</td>
<td></td>
<td></td>
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<tr>
<td>B cinerea</td>
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<tr>
<td>Candida</td>
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</tr>
<tr>
<td>C herb</td>
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<tr>
<td>M race</td>
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<td>T rubrum</td>
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<td>Omp p6</td>
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<td></td>
</tr>
<tr>
<td>Timothy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mugwort</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ragweed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.4 IL-10 Grouped: Atopic vs Nonatopic Asthma

Nonatopic Asthma (n = 25)
Atopic Asthma (n = 16)
Figure 5.5 IL-10: Eosinophilic vs Noneosinophilic

Stimulus

pg/mL

0
50
100
150
200

Eosinophilic (n = 31)
Noneosinophilic (n = 10)
Figure 5.6 IL-10 Grouped: Eosinophilic vs Noneosinophilic

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Stimulus

pg/mL

Eosinophilic (n = 31)
Noneosinophilic (n = 10)
Figure 5.7 IL-10: Neutrophilic vs Nonneutrophilic

Stimulus

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Figure 5.8 IL-10 Grouped: Neutrophilic vs Nonneutrophilic

Stimulus

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

pg/mL

Nonneutrophilic (n = 35)
Neutrophilic (n = 6)
Figure 6.1 IFN-gamma: Asthma vs Normal

Stimulus

Asthma (n = 38)
Normal Controls (n = 8)
Figure 6.2 IFN-gamma Grouped: Asthma vs Normal

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Asthma (n = 40)
Normal Controls (n = 8)

* p = 0.0388

Stimulus

pg/mL
Figure 6.3 IFN-gamma: Atopic vs Nonatopic Asthma

- Nonatopic Asthma (n = 25)
- Atopic Asthma (n = 13)
Figure 6.4 IFN-gamma Grouped: Atopic vs Nonatopic Asthma

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus vs pg/mL

- Nonatopic Asthma (n = 25)
- Atopic Asthma (n = 13)
Figure 6.5 IFN-gamma: Eosinophilic vs Noneosinophilic

Stimulus

- pg/mL

Eosinophilic (n = 28)

Noneosinophilic (n = 10)
Figure 6.6 IFN-gamma Grouped: Eosinophilic vs Noneosinophilic

Eosinophilic (n = 28)

Noneosinophilic (n = 10)

Stimulus

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

pg/mL
Figure 6.7 IFN-gamma: Neutrophilic vs Nonneutrophilic

Stimulus

- Nonneutrophilic (n = 32)
- Neutrophilic (n = 6)
Figure 6.8 IFN-gamma Grouped: Neutrophilic vs Nonneutrophilic

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Stimulus

pg/mL

Nonneutrophilic (n = 32)
Neutrophilic (n = 6)
Figure 7.1 All Cytokines: Asthma

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

Stimulus (pg/mL)
Figure 7.2 All Cytokines: Normal

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

Stimulus

0 200 400 600 800

pg/mL
Figure 7.3 All Cytokines: Nonatopic Asthma

Stimulus

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma
Figure 7.4 All Cytokines: Atopic Asthma

Stimulus

pg/mL

0 200 400 600 800

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

FSDM L dest
FSDM A siro
HDM D pteron
HDM D farinae
HDM E maynei
C Roach Peri Am
c C Roach B Ger
Cap Ep F catus
Cat Dand
Dog Dand
Ham Ep
Mouse Ep
Alternaria
A fum
A pull
B. cinerea
Candida
C. per
E. floc
F. solani
H. solani
M. canis
M. race
P. notatum
P. betae
S. cervis
T. rubrum
Psp A1
Psp A2
Psp C
Omp p6
Timothy
Mugwort
Ragweed
Figure 7.5 All Cytokines: Eosinophilic Asthma
Figure 7.6 All Cytokines: Noneosinophilic Asthma

- IL-5
- IL-10
- IL-17A
- IL-23
- IL-10
- IFN-gamma

Figure 7.7 All Cytokines: Neutrophilic Asthma

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

Stimulus: pg/mL

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Figure 7.8 All Cytokines: Nonneutrophilic Asthma

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 8.1 Skin Prick Test: Asthma

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>IL-5</th>
<th>IL-17A</th>
<th>IL-23</th>
<th>IL-10</th>
<th>IFN-gamma</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDM D pteron</td>
<td>400</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HDM D farinae</td>
<td>800</td>
<td>600</td>
<td>400</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td>C Roach Peri Am</td>
<td>600</td>
<td>400</td>
<td>200</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>C Roach B Ger</td>
<td>400</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cat Ep F catus</td>
<td>600</td>
<td>400</td>
<td>200</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Dog Dand</td>
<td>400</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alternaria</td>
<td>200</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A fum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Candida</td>
<td>600</td>
<td>400</td>
<td>200</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>C herb</td>
<td>400</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C sphaer</td>
<td>200</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Timothy</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mugwort</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Ragweed</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 8.2 Skin Prick Test: Normal

- HDM D pteron
- HDM D farinae
- C Roach Peri Am
- C Roach B Ger
- Cat Dand
- Dog Dand
- Alternaria
- A fum
- Candida
- C herb
- C sphaer
- Timothy
- Mugwort
- Ragweed

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 8.3 Skin Prick Test: Nonatopic Asthma

HDM D pteron
HDM D farinae
C Roach Peri Am
C Roach B Ger
Cap Ep F catus
Cat Dand
Dog Dand
Alternaria
A fum
Candida
C herb
C sphaer
Timothy
Mugwort
Ragweed

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 8.4 Skin Prick Test: Atopic Asthma

HDM D pteron
HDM D farinae
C Roach Peri Am
C Roach B Ger
Cap Ep F catus
Dog Dand
Alternaria
A fum
Candida
C herb
C sphaer
Timothy
Mugwort
Ragweed

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

0
200
400
600
800
Figure 8.5 Skin Prick Test: Eosinophilic Asthma

HDM D pteron
HDM D farinae
C Roach Peri Am
C Roach B Ger
Cap Ep F catus
Cat Dand
Dog Dand
Alternaria
A fum
Candida
C herb
C sphaer
Timothy
Mugwort
Ragweed

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 8.6 Skin Prick Test: Noneosinophilic Asthma

Stimuli:
- HDM D pteron
- HDM D farinae
- C Roach Peri Am
- C Roach B Ger
- Cap Ep F catus
- Cat Dand
- Dog Dand
- Alternaria
- A fum
- Candida
- C herb
- C sphaer
- Timothy
- Mugwort
- Ragweed

Levels of IL-5, IL-17A, IL-23, IL-10, and IFN-gamma are indicated by the bars.
Figure 8.7 Skin Prick Test: Neutrophilic Asthma

HDM D pteron
HDM D farinae
C Roach Peri Am
C Roach B Ger
Cap Ep F catus
C oculis
Timothy
Mugwort
Ragweed
0
200
400
600
800
IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Stimulus
pg/mL
Figure 8.8 Skin Prick Test: Nonneutrophilic Asthma

- HDM D pteron
- HDM D farinae
- C Roach Peri Am
- C Roach B Ger
- Cap Ep F catus
- Cat Dand
- Dog Dand
- Alternaria
- A fum
- C sphaer
- Timothy
- Mugwort
- Ragweed

Stimulus

(pg/mL)

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 9.1 Delta Grouped: Steroids

- 0-500 mg ICS (n = 2)
- 500-1000 mg ICS (n = 9)
- > 1000 mg ICS (n = 5)
- 0-10 mg Prednisone (n = 10)
- > 10 mg Prednisone (n = 11)

Stimulus:
- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds
Figure 9.2 IL-5 Grouped: Steroids

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

- 0-500 mg ICS (n = 2)
- 500-1000 mg ICS (n = 9)
- > 1000 mg ICS (n = 5)
- 0-10 mg Prednisone (n = 12)
- > 10 mg Prednisone (n = 11)

- p = 0.0364
- p = 0.0358
- p = 0.0382
- p = 0.0382
- p = 0.0402
Figure 9.3 IL-17A Grouped: Steroids

- 0-500 mg ICS (n = 2)
- 500-1000 mg ICS (n = 9)
- > 1000 mg ICS (n = 5)
- 0-10 mg Prednisone (n = 12)
- > 10 mg Prednisone (n = 11)
Figure 9.4 IL-23 Grouped: Steroids

- 0-500 mg ICS (n = 2)
- 500-1000 mg ICS (n = 9)
- > 1000 mg ICS (n = 5)
- 0-10 mg Prednisone (n = 12)
- > 10 mg Prednisone (n = 11)
Figure 9.5 IL-10 Grouped: Steroids

- 0-500 mg ICS (n = 2)
- 500-1000 mg ICS (n = 9)
- > 1000 mg ICS (n = 5)
- 0-10 mg Prednisone (n = 11)
- > 10 mg Prednisone (n = 11)

Stimulus

pg/mL
Figure 9.6 IFN-gamma Grouped: Steroids

- 0-500 mg ICS (n = 2)
- 500-1000 mg ICS (n = 9)
- > 1000 mg ICS (n = 5)
- 0-10 mg Prednisone (n = 9)
- > 10 mg Prednisone (n = 10)

Stimulus

pg/mL
Average allergen-induced IL-5 production by PBMCs from eosinophilic asthmatic patients compared across 5 steroid treatment groups and analyzed by one-way ANOVA.

**Figure 9.7 IL-5: Steroid Dosage**

- 0-500 mg ICS
- 500-1000 mg ICS
- $> 1000$ mg ICS
- 0-10 mg Prednisone
- $> 10$ mg Prednisone

$$p = 0.0001$$  
$$r^2 = 0.5934$$
APPENDIX III: INDIVIDUAL PATIENT RESULTS

Contains:
1. Nonatopic Asthmatic Patients
2. Atopic Asthmatic Patients
3. Normal Controls
Figure 10.1 Nonatopic Asthmatic Patient 1 - Noneosinophilic

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

0
100
200
300
400
500

FSDM L dest
FSDM A siro
HDM D pteron
HDM D farinae
HDM E maynei
C Roach Peri Am
C Roach B Ger
Cap Ep
F catus
Cat Dand
Dog Dand
Rat Ep
Ham Ep
Mouse Ep
Alternaria
A fum
B cinerea
Candida
C sphaer
E floc
F solani
H solani
M canis
M race
P notatum
P betae
S cervis
T rubrum
Psp A1
Psp A2
Psp C
Omp P8
Timothy
Mugwort
Ragweed
Figure 10.2 Nonatopic Asthmatic Patient 1 - Noneosinophilic

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

pg/mL

0
100
200
50
100
150
200

140

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Figure 10.3: Nonatopic Asthmatic Patient 2 - Eosinophilic

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 10.4 Nonatopic Asthmatic Patient 2 - Eosinophilic

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 10.5 Nonatopic Asthmatic Patient 3 - Eosinophilic

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

pg/mL

Stimulus

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Figure 10.6 Nonatopic Asthmatic Patient 3 - Eosinophilic

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

<table>
<thead>
<tr>
<th>IL-5</th>
<th>IL-17A</th>
<th>IL-23</th>
<th>IL-10</th>
<th>IFN-gamma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

pg/mL
Figure 10.7 Nonatopic Asthmatic Patient 4 - Eosinophilic

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 10.8 Nonatopic Asthmatic Patient 4 - Eosinophilic

<table>
<thead>
<tr>
<th>Mites/Insects</th>
<th>Animal Dander/Epithelia</th>
<th>Fungi</th>
<th>Microbial</th>
<th>Grass/Weeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-5</td>
<td>IL-17A</td>
<td>IL-23</td>
<td>IL-10</td>
<td>IFN-gamma</td>
</tr>
</tbody>
</table>

Stimulus

pg/mL

0  50  100  150  200  250  300

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 10.9 Noneosinophilic Asthmatic Patient 5 - Neutrophilic

Chloroform

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 10.10 Noneosinophilic Asthmatic Patient 5 - Neutrophilic

![Graph showing cytokine levels](image)

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

Stimulus: Mites/Insects, Animal Dander/Epithelia, Fungi, Microbial, Grass/Weeds
Figure 10.11 Nonatopic Asthmatic Patient 6 - Eosinophilic

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

Stimulus

pg/mL
Figure 10.12 Nonatopic Asthmatic Patient 6 - Eosinophilic

The diagram shows the cytokine levels in response to various stimuli. The x-axis represents different stimuli: Mites/Insects, Animal Dander/Epithelia, Fungi, Microbial, and Grass/Weeds. The y-axis represents the stimulus concentration in pg/mL, ranging from 0 to 200.

The cytokines measured include IL-5, IL-17A, IL-23, IL-10, and IFN-gamma. Each stimulus has a different color code:
- IL-5: Dark line
- IL-17A: Light line
- IL-23: Grey line
- IL-10: Crossed grey line
- IFN-gamma: Crossed light grey line

The levels of each cytokine vary across different stimuli, indicating a response to the specific allergens present.
Figure 10.13 Nonatopic Asthmatic Patient 7 - Eosinophilic

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

Stimulus (pg/mL)
**Figure 10.14** Nonatopic Asthmatic Patient 7 - Eosinophilic

The chart displays the levels of various cytokines in response to different stimuli.

- **Mites/Insects**
- **Animal Dander/Epithelia**
- **Fungi**
- **Microbial**
- **Grass/Weeds**

The y-axis represents concentration in pg/mL, ranging from 0 to 300.

The chart shows the following cytokines with distinct markers:
- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

Stimuli are shown at the bottom of the chart.
Figure 10.15 Nonatopic Asthmatic Patient 8 - Noneosinophilic

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 10.16 Nonatopic Asthmatic Patient 8 - Noneosinophilic

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus:
- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

pg/mL
Figure 10.17 Nonatopic Asthmatic Patient 9 - Eosinophilic

Stimulus

pg/mL

0 200 400 600 800 1000

IL-5

IL-17A

IL-23

IL-10

IFN-gamma
Figure 10.18 Nonatopic Asthmatic Patient 9 - Eosinophilic

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 10.19 Nonatopic Asthmatic Patient 10 - Eosinophilic

Stimulus

pg/mL

0 200 400 600

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 10.20 Nonatopic Asthmatic Patient 10 - Eosinophilic

Stimulus

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

pg/mL
Figure 10.21 Nonatopic Asthmatic Patient 11 - Neutrophilic

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

Stimulus (pg/mL)
Figure 10.22 Nonatopic Asthmatic Patient 11 - Neutrophilic

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 10.23 Nonatopic Asthmatic Patient 12 - Eosinophilic

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

0
200
400
600
800

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 10.24 Nonatopic Asthmatic Patient 12 - Eosinophilic

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>IL-5</th>
<th>IL-17A</th>
<th>IL-23</th>
<th>IL-10</th>
<th>IFN-gamma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mites/Insects</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Animal Dander/Epithelia</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Fungi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grass/Weeds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

pg/mL

0 50 100 150 200 250 IL-5 IL-17A IL-23 IL-10 IFN-gamma

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Figure 10.25 Nonatopic Asthmatic Patient 13 - Neutrophilic

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 10.26 Nonatopic Asthmatic Patient 13 - Neutrophilic

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

pg/mL

0 100 200 300 400
Figure 10.27 Nonatopic Asthmatic Patient 15 - Eosinophilic/Neutrophilic

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 10.28 Nonatopic Asthmatic Patient 15 - Eosinophilic/Neutrophilic

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 10.29 Nonatopic Asthmatic Patient 16 - Eosinophilic

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 10.30 Nonatopic Asthmatic Patient 16 - Eosinophilic

Stimulus

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

pg/mL
0
50
100
150
200

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Figure 10.31 Nonatopic Asthmatic Patient 17 - Eosinophilic

![Graph showing cytokine levels in response to various stimuli.](image)

- IL-5
- IL-17A
- IL-23

Stimulus:
- PG/mL
- FSDM L dest
- FSDM A sir
- HDM D pteron
- HDM D farinae
- HDM E maynei
- C Roach Peri Am
- C Roach B Ger
- Cap Ep F cat
- Cat Dand
- Dog Dand
- Rat Ep
- Ham Ep
- Mouse Ep
- Alternaria
- A fum
- A pull
- B cinerea
- Candida
- C sphaer
- C floc
- F solani
- H solani
- M canis
- M race
- P notatum
- P betae
- S cervis
- T rubrum
- Pap A1
- Pap A2
- Pap C
- Omp p6
- Timothy
- Mugwort
- Ragweed

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Figure 10.32 Nonatopic Asthmatic Patient 17 - Eosinophilic

Stimulus

pg/mL

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

IL-5
IL-17A
IL-23
Figure 10.33 Nonatopic Asthmatic Patient 18 - Eosinophilic

Stimulus

0 500 1000 1500 2000
pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

FSDM L dest
FSDM A siro
HDM D pteron
HDM D farinae
HDM E maynei
C Roach Peri Am
C Roach B Ger
Cap Ep F catus
Cat Dand
Dog Dand
Rat Ep
Ham Ep
Mouse Ep
Alternaria
A fum
A pull
B cinerea
Candida
C sphaer
E floc
F solani
H solani
M canis
M race
P notatum
P betae
S cervis
T rubrum
Psp A1
Psp A2
Psp C
Omp p6
Timothy
Mugwort
Ragweed

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 10.34 Nonatopic Asthmatic Patient 18 - Eosinophilic

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

Stimulus (pg/mL)
Figure 10.35 Nonatopic Asthmatic Patient 20 - Eosinophilic

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 10.36 Nonatopic Asthmatic Patient 20 - Eosinophilic

Stimulus

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

pg/mL
Figure 10.37 Nonatopic Asthmatic Patient 21 - Eosinophilic

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 10.38 Nonatopic Asthmatic Patient 21 - Eosinophilic

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 10.39 Nonatopic Asthmatic Patient 22 - Nonosinophilic

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 10.40 Nonatopic Asthmatic Patient 22 - Nonosinophilic

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

pg/mL

Stimulus

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 10.41 Nonatopic Asthmatic Patient 23 - Eosinophilic

Stimulus

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Figure 10.42 Nonatopic Asthmatic Patient 23 - Eosinophilic

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 10.43 Nonatopic Asthmatic Patient 24 - Neutrophilic IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Stimulus
pg/mL
0
500
1000
1500
2000
FSDM L dest
FSDM A siro
HDM D pteron
HDM D farinae
HDM E maynei
C Roach Peri Am
C Roach B Ger
Cap Ep F catus
Cat Dand
Dog Dand
Rat Ep
Ham Ep
Mouse Ep
Alternaria
A tum
A pull
B chnera
Candida
C sphaer
C solani
C fum
C pull
B cinerea
C herbi
C sphaer
C solani
M canis
M race
P notatum
P betae
S cervis
T rubrum
Psp A1
Psp A2
Psp C
Omp p6
Timothy
Mugwort
Ragweed
Figure 10.44 Nonatopic Asthmatic Patient 24 - Neutrophilic

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>IL-5</th>
<th>IL-17A</th>
<th>IL-23</th>
<th>IL-10</th>
<th>IFN-gamma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mites/Insects</td>
<td></td>
<td></td>
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<td></td>
</tr>
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<td>Animal Dander/Epithelia</td>
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<td></td>
<td></td>
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<tr>
<td>Fungi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grass/Weeds</td>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

pg/mL
Figure 10.45 Nonatopic Asthmatic Patient 25 - Eosinophilic

Stimulus

pg/mL

0
200
400
600
800
1000

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 10.46 Nonatopic Asthmatic Patient 25 - Eosinophilic

Stimulus

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

pg/mL
Figure 10.47 Nonatopic Asthmatic Patient 26 - Eosinophilic

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 10.48 Nonatopic Asthmatic Patient 26 - Eosinophilic

Stimulus

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

pg/mL

0
50
100
150

Mites/Insects
Animal Dander/Epithelia
Fungi
Figure 10.50 Nonatopic Asthmatic Patient 27 - Eosinophilic

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 10.51 Nonatopic Asthmatic Patient 27 - Eosinophilic

- **Mites/Insects**
- **Animal Dander/Epithelia**
- **Fungi**
- **Microbial**
- **Grass/Weeds**

Legend:
- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

Stimulus

pg/mL

0 50 100 150 200 250

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 10.52 Nonatopic Asthmatic Patient 28 - Eosinophilic

Stimulus: [list of stimuli]

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 10.53 Nonatopic Asthmatic Patient 28 - Eosinophilic Mites/Insects Animal Dander/Epithelia Fungi Microbial Grass/Weeds 0 50 100 150 200 250 IL-5 IL-17A IL-23 IL-10 IFN-gamma Stimulus pg/mL
Figure 11.1 Atopic Asthmatic Patient 1 - Neutrophilic
Allergies: Ragweed, mold, dust mite, peanut, birch tree

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 11.2 Atopic Asthmatic Patient 1 - Neutrophilic
Allergies: Ragweed, mold, dust mite, peanut, birch tree

Stimulus

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

pg/mL

0 10 20 30 40
Figure 11.3 Atopic Asthmatic Patient 2- Eosinophilic
Allergies: cat, dust mite, grass, alternaria

- FSDM L dest
- FSDM A siro
- HDM D fanae
- HDM E maynei
- C. Roach B ger
- C. Roach F cura
- Cat Dand
- Dog Dand
- Rat Ep
- Mouse Ep
- Alternaria
- A. fum
- A. pull
- B. cinerea
- C. herba
- C. sphaer
- E. floc
- H. solani
- M. canis
- M. raci
- P. notatum
- P. betae
- S. cervis
- T. rubrum
- Psp A
- Psp A2
- Omp P8
- Timothy
- Mugwort
- Ragweed

Stimulus

pg/mL

0
500
1000
1500
IL-5
IL-17A
IL-10
IFN-gamma
IL-23

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Figure 11.4 Atopic Asthmatic Patient 2 - Eosinophilic Allergies: cat, dust mite, grass, alternaria

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Stimulus

pg/mL

IL-5
IL-17A
IL-10
IFN-gamma
IL-23

Allergies: cat, dust mite, grass, alternaria
Figure 11.5 Atopic Asthmatic Patient 4 - Eosinophilic Allergies: dust mite

Stimulus

pg/mL

0
200
400
600
800
1000

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

Stimulus
Figure 11.6 Atopic Asthmatic Patient 4 - Eosinophilic
Allergies: dust mite

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 11.7 Atopic Asthmatic Patient 5 - Eosinophilic Allergies: dust mite, mold

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 11.8 Atopic Asthmatic Patient 5 - Eosinophilic Allergies: dust mite, mold

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Stimulus

pg/mL

0
100
200
300

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 11.9 Atopic Asthmatic Patient 6 - Noneosinophilic

Allergies: Dust mite, ragweed, aspergillus, trees, grass, alternaria, cat, horse, dictyoptera

The figure depicts a bar chart showing the levels of various cytokines (IL-5, IL-17A, IL-23, IL-10, IFN-gamma) in response to different stimuli in a noneosinophilic atopic asthmatic patient. The x-axis represents the stimulus, while the y-axis shows the levels of cytokines in pg/mL.
Figure 11.10 Atopic Asthmatic Patient 6 - Noneosinophilic
Allergies: Dust mite, ragweed, aspergillus, trees, grass, alternaria, cat, horse, dictyoptera

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

Stimulus

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

pg/mL
Figure 11.11 Atopic Asthmatic Patient 7 - Eosinophilic Allergies: dust mite

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 11.12 Atopic Asthmatic Patient 7 - Eosinophilic Allergies: dust mite

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

Stimulus: pg/mL

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds
Figure 11.13 Atopic Asthmatic Patient 8 - Eosinophilic Allergies: dust mite, mold

- FSDM L dest
- FSDM A siro
- HDM D pteron
- HDM D farinae
- HDM E maynei
- C Roach Peri Am
- C Roach B Ger
- Cap Ep F catus
- Cat Dand
- Dog Dand
- Rat Ep
- Ham Ep
- Alternaria
- A fum
- A pull
- B cinerea
- A1
- Psp A2
- Psp C
- Omp p6
- Timothy
- Mugwort
- Ragweed

Stimulus: pg/mL

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma
Figure 11.14 Atopic Asthmatic Patient 8 - Eosinophilic Allergies: dust mite, mold

<table>
<thead>
<tr>
<th>Allergens</th>
<th>IL-5</th>
<th>IL-17A</th>
<th>IL-23</th>
<th>IL-10</th>
<th>IFN-gamma</th>
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<tbody>
<tr>
<td>Mites/Insects</td>
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<td>Animal Dander/Epithelia</td>
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<td>Fungi</td>
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<tr>
<td>Microbial</td>
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</tr>
<tr>
<td>Grass/Weeds</td>
<td></td>
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</tr>
</tbody>
</table>

Stimulus: pg/mL
Figure 11.15 Atopic Asthmatic Patient 9 - Noneosinophilic
Allergens: dust mite, pollens

- FSDM L dest
- FSDM A siro
- HDM D pteron
- HDM D farinae
- C Roach Peri Am
- C Roach B Ger
- Cap Ep F catus
- Cat Dand
- Dog Dand
- Rat Ep
- Ham Ep
- Altararia
- A fum
- A pull
- B cinerea
- Candida
- C sphaer
- E floc
- F solani
- H solani
- M canis
- M race
- P notatum
- P betae
- S cervis
- T rubrum
- Psp A1
- Psp A2
- Psp C
- Omp p6
- Timothy
- Mugwort
- Ragweed

Stimulus

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

pg/mL
Figure 11.16 Atopic Asthmatic Patient 9 - Noneosinophilic
Allergens: dust mite, pollens

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

pg/mL

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma
Figure 11.17 Atopic Asthmatic Patient 10 - Eosinophilic
Allergies: Alternaria, cat, horse, feathers, grass

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 11.18 Atopic Asthmatic Patient 10 - Eosinophilic Allergies: Alternaria, cat, horse, feathers, grass

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus vs. pg/mL:
- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

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Figure 11.19 Atopic Asthmatic Patient 11 - Eosinophilic Allergies: grass, peanuts, shellfish, animals

![Graph showing cytokine levels](image-url)

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

Stimulus vs. Cytokine Levels (pg/mL)

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Figure 11.20 Atopic Asthmatic Patient 11 - Eosinophilic Allergies: grass, peanuts, shellfish, animals

Stimulus

pg/mL

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 11.21 Atopic Asthmatic Patient 12 - Eosinophilic Allergies: dust mite

Stimulus

pg/mL

0
1000
2000
3000

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 11.22 Atopic Asthmatic Patient 12 - Eosinophilic Allergies: dust mite

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 11.23 Atopic Asthmatic Patient 13 - Eosinophilic Allergies: cat, dog, horse

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10

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**Figure 11.24** Atopic Asthmatic Patient 13 - Eosinophilic Allergies: cat, dog, horse

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus (pg/mL): IL-5, IL-17A, IL-23, IL-10
Figure 11.25 Atopic Asthmatic Patient 14 - Eosinophilic
Allergies: grass, dust, milk

pg/mL

Stimulus
Figure 11.26 Atopic Asthmatic Patient 14 - Eosinophilic Allergies: grass, dust, milk

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus vs. pg/mL:
- IL-5
- IL-17A
- IL-23
- IL-10
Figure 11.27 Atopic Asthmatic Patient 15 - Eosinophilic
Allergies: d. far, d. pter, grass, ragweed
Figure 11.28 Atopic Asthmatic Patient 15 - Eosinophilic Allergies: d. far, d. pter, grass, ragweed

Stimulus

pg/mL

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

IL-5
IL-17A
IL-23
IL-10
Figure 11.29 Atopic Asthmatic Patient 16 - Eosinophilic Allergies: dust mite, dictoptera

Stimulus

pg/mL

0
10
20
30
40

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

FSDM L dest
FSDM A siro
HDM D pteron
HDM D farinae
HDM E maynei
Roach Peri Am
Roach B Ger
Cap Ep F cat
Cat Dand
Dog Dand
Rat Dand
Mice Ep
Mouse Ep
Alternaria
A fum
A pull
B cinerea
C Candida
C sphaer
C aphano
E floc
F solani
H solani
M canis
M race
P notatum
P betae
P Poot
P fam
S cerevis
t
T rubrum
Psp A1
Psp A2
Psp C
Omp p6
Timothy
Mugwort
Ragweed

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Figure 11.30 Atopic Asthmatic Patient 16 - Eosinophilic Allergies: dust mite, dictoptera

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 11.31 Atopic Asthmatic Patient 17 - Eosinophilic Allergies: cat, horse, feathers, dust mite

Stimulus

pg/mL

0 200 400 600 800

IL-5

IL-17A

IL-23

IL-10

IFN-gamma
Figure 11.32 Atopic Asthmatic Patient 17 - Eosinophilic
Allergies: cat, horse, feathers, dust mite

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

Stimulus (pg/mL)
Figure 12.2 Normal Control 7

- Mites/Insects
- Animal Dander/Epithelia
- Fungi
- Microbial
- Grass/Weeds

Stimulus

pg/mL

IL-5
IL-17A
IL-10
IFN-gamma
Figure 12.3 Normal Control 9

Stimulus pg/mL

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

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Figure 12.4 Normal Control 9

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

Stimulus: Mites/Insects, Animal Dander/Epithelia, Fungi, Microbial, Grass/Weeds

(pg/mL)
Figure 12.5 Normal Control 10

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

0
500
1000

FSDM L dest
FSDM A siro
HDM D pteron
HDM D farinae
HDM E maynei
C Roach Peri Am
C Roach B Ger
Cap Ep F catus
Cat Dand
Dog Dand
Rat Ep
Ham Ep
Mouse Ep
Alternaria
A fum
A pull
B cinerea
Candida
C herb
C sphaer
E floc
F solani
H solani
M canis
M race
P notatum
P betae
S cervis
T rubrum
Psp A1
Psp A2
Psp C
Omp p6
Timothy
Mugwort
Ragweed
Figure 12.6 Normal Control 10

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 12.7 Normal Control 11

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

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Figure 12.8 Normal Control 11

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

0
50
100
150
pg/mL

Stimulus

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 12.9 Normal Control 12

Stimulus

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma

pg/mL

0 500 1000 1500
Figure 12.10 Normal Control 12

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Stimulus

pg/mL

- IL-5
- IL-17A
- IL-23
- IL-10
- IFN-gamma
Figure 12.12 Normal Control 13

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

0
50
100
150
200

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 12.13 Normal Control 14

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

FSDM L dest
FSDM A siro
HDM D pteron
HDM D farinae
HDM E maynei
C Roach Peri Am
C Roach B Ger
Cap Ep F catus
Cat Dand
Dog Dand
Rat Ep
Ham Ep
Mouse Ep
Alternaria
A fum
A pull
B cinerea
Candida
C herb
C sphaer
E floc
F solani
H solani
M canis
M race
P notatum
P betae
S cervis
T rubrum
Psp A1
Psp A2
Omp p6
Timothy
Mugwort
Ragweed

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Figure 12.14 Normal Control 14

Stimulus

pg/mL

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

IL-5
IL-17A
IL-23
IL-10
IFN-gamma

0
100
200
300
400
Figure 12.15 Normal Control 15

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
Figure 12.16 Normal Control 15

Mites/Insects
Animal Dander/Epithelia
Fungi
Microbial
Grass/Weeds

Stimulus

pg/mL

IL-5
IL-17A
IL-23
IL-10
IFN-gamma
REFERENCES


