# A BRIEF OVERVIEW OF LUNG DISEASE CAUSED BY ENVIRONMENTAL MICROORGANISMS, *ASPERGILLUS FUMIGATUS* AND *SACCHAROPOLYSPORA RECTIVIRGULA*

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**Title**

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**MASTER OF SCIENCE**

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**ABSTRACT**

People often take breathing and respiratory health for granted, but our lungs are constantly inundated with damaging agents, making them highly vulnerable to injury and infection. Globally, respiratory diseases are a leading cause of morbidity, mortality, and disability. Many respiratory diseases are preventable; however, avoidance of ubiquitous microorganisms is not realistic. The aim of this paper is to discuss how two environmental microorganisms, *Aspergillus fumigatus* and *Saccharopolyspora rectivirgula*, affect cellular activity and cause lung disease. Prevention, control, and cure of respiratory disease is the ultimate goal and understanding the mechanisms of disease progression is the first step.

**ACKNOWLEDGEMENTS**

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## DEDICATION

This is dedicated to my daughter, family, and friends. Without whom, none of this would be possible! Well, it might have been possible, just not worth it 😊

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| | |
|---|---|
| Ab | Antibody |
| Ag | Antigen |
| AHR | Airway hyperresponsiveness |
| AM | Alveolar macrophage |
| APC | Antigen presenting cell |
| BAL | Bronchoalveolar lavage |
| CD | Cluster of differentiation |
| COPD | Chronic obstructive pulmonary disease |
| CTL | Cytotoxic T lymphocyte |
| DAMP | Damage-associated molecular pattern |
| DALY | Disability-life adjusted year |
| DC | Dendritic cell |
| ECM | Extracellular matrix |
| ECP | Eosinophil cationic protein |
| EDN | Eosinophil-derived neurotoxin |
| EPO | Eosinophil peroxidase |
| $FEV_1$ | Forced expiratory volume |
| FLD | Farmer's lung disease |
| FVC | Force vital capacity |
| G-CSF | Granulocyte-colony stimulating factor |
| GGO | Ground glass opacity |
| GM-CSF | Granulocyte-macrophage colony-stimulating factor |
| HRCT | High-resolution computed tomography |
| HP | Hypersensitivity pneumonitis |
| HSC | Hematopoietic stem cell |
| IFN-γ | Interferon gamma |
| Ig | Immunoglobulin |

IL ..... Interleukin

IM ..... Interstitial macrophage

IPF ..... Idiopathic pulmonary fibrosis

LAMA ..... Long-acting muscarinic antagonists

LPS ..... Lipopolysaccharide

LT ..... Leukotriene

MAC ..... Macrophage

MBP ..... Major basic protein

MCP ..... Mast cell protease

NET ..... Neutrophilic extracellular trap

NKC ..... Natural killer cell

PRR ..... Pattern recognition receptor

ROI ..... Reactive oxygen intermediate

ROS ..... Reactive oxygen species

SABA ..... Short-acting beta-2 agonist

SCF ..... Stem cell factor

$T_H$ ..... T-helper

TLR ..... Toll-like receptor

TNF ..... Tumor necrosis factor

TSLP ..... Thymic stromal lymphopoietin

## INTRODUCTION

Because of the continuous inhalation of particulate matter, chemicals, and infectious microbes, the lung is most susceptible to infection and injury. The Forum of the International Respiratory Society has recognized five major lung issues, termed the “big five”, which includes chronic obstructive pulmonary disease (COPD), asthma, acute lower respiratory tract infections, tuberculosis, and lung cancer.[1] In 2019, approximately 334 million people suffered from asthma, globally.[2] It is the most common chronic childhood disease, affecting 14% of children worldwide and prevalence within this group continues to rise.[3] Lower respiratory tract infections cause an estimated 4 million deaths, more importantly infections in childhood predispose these patients to chronic lung problems later in life.[4] Disability-adjusted life-years (DALYs), a measurement of estimated years lost due to sickness, disability, or premature death, caused by respiratory diseases was 10% of the total 24.6 billion DALYs estimated in 2015.[5]

There are many allergic conditions involving the lung and knowing the basic immunological reactions that are taking place is paramount in our understanding of allergic lung diseases. Insight into the underlying mechanisms of allergic reactions taking place in the lung can help our knowledge of lung diseases, such as hypersensitivity pneumonitis (HP) and allergic asthma. With the increase in the number of immunocompromised people, diseases caused by both bacteria and fungi continue to rise.[6] To diminish the damage caused by bacterial and fungal infections, the body utilizes sophisticated defense mechanisms using both the innate and adaptive immune response to fight off foreign invaders.[7]

People are exposed to microbes that are ubiquitous in nature, but not all develop the diseases. The purpose of this paper is to provide a brief overview of two environmental microorganisms, *Aspergillus fumigatus* and *Saccharopolyspora rectivirgula*, how they affect the cellular environment in the lung, and how disease progression occurs in the lungs.

## MICROORGANISMS

### Aspergillus fumigatus

*Aspergillus fumigatus* (*A. fumigatus*) is a ubiquitous, saprophytic fungus that develops and releases airborne, hydrophobic conidia into the atmosphere. Humans inhale several hundreds of these conidia daily.[8] It is an opportunistic pathogen that is implicated in a number of human diseases, including aspergilloma, allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA), invasive aspergillosis (IA), sinusitis, otomycosis, ocular infections, central nervous system (CNS) infection, osteomyelitis, cutaneous aspergillosis, endocarditis, and urinary tract infection.[9,10]

*A. fumigatus* has several unique characteristics that make it a highly successful pathogen. This includes: (a) the ability to produce thousands of airborne conidia, (b) capability to grow in unfavorable environments, (c) conidia can reach the alveolar space dues to its small size (2 – 3μm), (d) use of extracellular enzymes to acquire nutrients in the lung environment, (e) evasion of recognition by host immune response (e.g. toll-like receptors) due to its structure, (f) metabolites such as gliotoxin and fumagillin that counteract host immune response. All of these properties have been areas of studying fungal-host relationships.[11]

Clearance of *A. fumigatus* conidia, in an immunocompetent person, is normally coordinated by the innate immune response. The immediate response to conidia is coordinated by epithelial cells and phagocytes (chiefly alveolar macrophages and neutrophils) and used to eliminate fungal conidia. Most conidia will never contact alveolar epithelium, as the mucociliary escalator of the bronchi, bronchioles, and nasopharynx will clear a majority of inhaled conidia.[12] If conidia are able to bypass mucociliary clearance, airway epithelial cells are able to play a key part in immune response by not only providing a physical barrier, but by producing cytokines, chemokines, and other proteins involved in the innate immune response; these include expression of pattern recognition receptors (PRRs) and secretion of pro-inflammatory effector proteins.[13] Alveolar macrophages (AMs) can take up and kill conidia by generation of reactive oxygen species (ROS) and/or phagosomal acidification. ROS generation is a response to swollen conidia that leads to recruitment of cytosolic proteins to the plasma membrane to form NADPH oxidase complex and initiates formation of the phagosome.[14] This immediate response leads to the initiation of the inflammatory response and recruitment of neutrophils followed by influx of monocytes,

dendritic cells, mast cells, eosinophils, and natural killer cells (Figure 1), all of which produce mediators that contribute to fungal clearance (Table 1).[15]

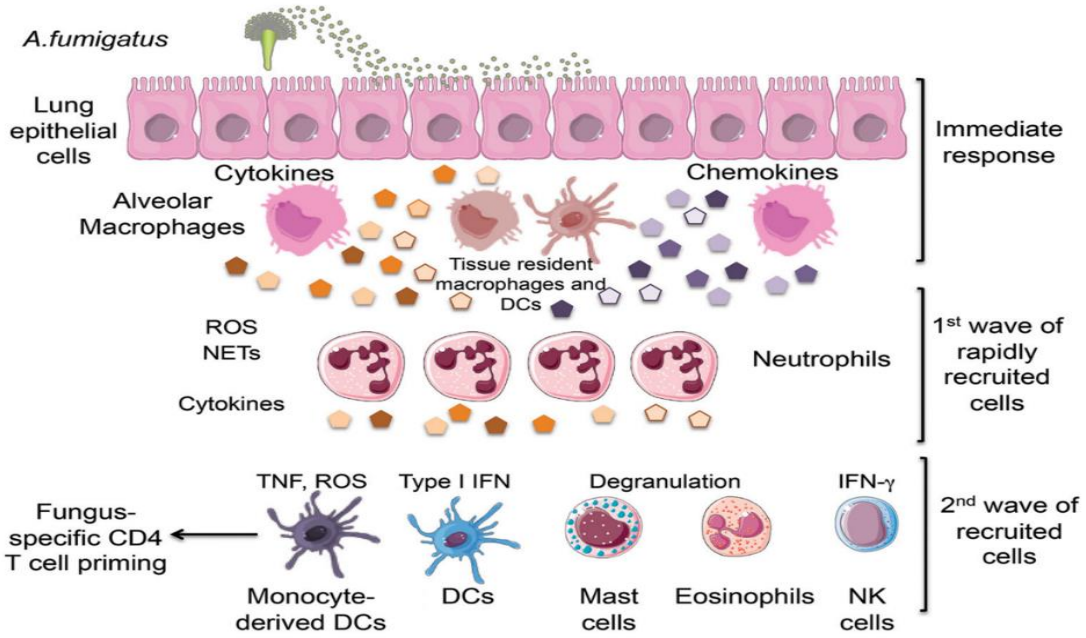



**Figure 1. Innate Response after Exposure to *A. fumigatus* Conidia.** The immediate response to conidia is orchestrated by epithelial cells and alveolar macrophages, which secrete cytokines and chemokines to initiate an inflammatory response and recruit neutrophils, respectively. This is followed by influx of monocytes, dendritic cells, mast cells, eosinophils, and natural killer cells that release mediators that aid in clearance of conidia. From "First Line of Defense: Innate Cell-Mediated Control of Pulmonary Aspergillosis" by Espinosa, V. and A. Rivera, 2016, Front Microbiol 7: 272, p. 4. CC BY.[15]

**Table 1. Summary of Innate Cell Function after *A. fumigatus* Infection**

| Cell type | Effector function |
|---|---|
| Epithelial cells | Barrier protection<br>Production of pro-inflammatory cytokines (TNF-α, IL-8) |
| Alveolar macrophages | ROS production and phagosomal acidification<br>Cytokine and chemokine production (e.g. CXCL1/2) |
| Neutrophils | ROS generation<br>NETosis<br>Release of proteases via degranulation |
| Monocytes | Differentiation into macrophages<br>Skew adaptive immune response to $T_H1$ |
| Dendritic cells | Production of IL-2<br>Antigen presentation |
| Mast cells | Degranulation – release of histamine and tryptase |
| Eosinophils | Degranulation – release of proteins |
| Natural killer cells | Release of perforin<br>Cytokine production (IFN-γ) |

[a]Adapted from "First Line of Defense: Innate Cell-Mediated Control of Pulmonary Aspergillosis" by Espinosa, V. and A. Rivera, 2016, Front Microbiol 7: 272, p. 2. CC BY.[15]

**Saccharopolyspora rectivirgula**

*Saccharopolyspora rectivirgula* (*S. rectivirgula*), formerly known as *Micropolyspora faeni*[16] and *Faenia rectivirgula*[17], is a Gram-positive, filamentous, aerobic, and thermophilic bacteria that produces short chains of spores on substrates and aerial mycelia (Figure 2). These spores range between 0.7 and 1.5μm in diameter.[18] This group usually has a high guanine (G), cytosine (C) content (G + C), as high as 70%, with *S. rectivirgula* having a G + C content of 68.9%.[19] *S. rectivirgula* belongs to the phylum *Actinobacteria* which is of economic importance, as agriculture and forestry depend on their contributions to soil systems. In soil, this phylum behaves like fungi, in that they decompose organic matter of dead organisms and often grow extensive mycelia.[20] *S. rectivirgula* was first isolated from moldy hay that had been baled in conditions containing >35% water and 50-65°C. Since then it has been found in a variety of substrates, including cereals, cotton bales, mushroom compost, and straw. *S. rectivirgula* is the chief causative agent of farmer's lung, a type of hypersensitivity pneumonitis, and is caused by inhalation of a large number of spores from moldy substrates.[21]

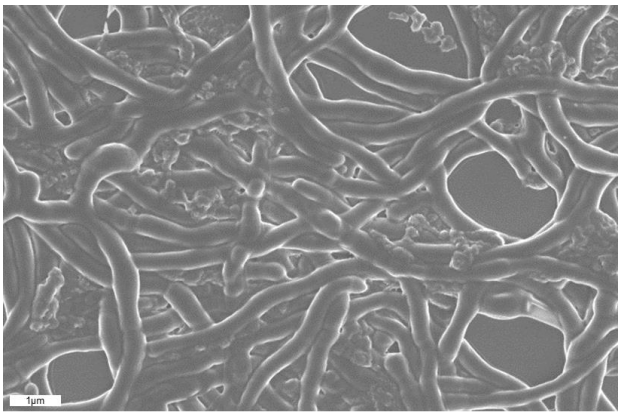



**Figure 2. SEM of *S. rectivirgula*.**
Scanning electron microscope image showing the filamentous nature of S. rectivirgula. 10,000x. Courtesy of NDSU Electron Microscopy Center.

There is still some question as to which elements of *S. rectivirgula* are the antigenic portion that causes disease. There have been studies indicating the extracellular enzymes associated with *S. rectivirgula* are able to act as allergens.[22] A study by Mundt, et al. tested which *S. rectivirgula* antigens reacted with $IgG_2$ antibodies from patients with farmer's lung disease. One-third of the patients' $IgG_2$ antibodies reacted to two major acidic proteins with molecular weights of 12 and 30kD and identical N-

terminus amino acid sequences of ADXDLLAQE.[23] Two-thirds of $IgG_2$ antibodies reacted against concanavalin A-binding glycoproteins containing mainly glucose, mannose, and galactose residues. Deglycosylation studies with the concanavalin-A binding portions showed that the majority of reactivity was to the carbohydrate regions of *S. rectivirgula*.[23]

Typically, bacteria will never reach the alveolar spaces in the lungs and clearance is similar to that of fungal conidia. It involves the mucociliary escalator, which is comprised of ciliated epithelium of the airways, located from the trachea to the bronchioles, and the mucus covering the epithelial surface. The mucociliary escalator functions by trapping foreign particles, like bacteria, in the mucus layer and transferring them out via coughing and ciliary beating.[24] Should bacteria reach the alveolar spaces, alveolar macrophages are the sentinel cells located at the alveolar-capillary interface. Alveolar macrophages are responsible for clearing any particles that managed to escape the mechanical immune system (i.e. mucociliary escalator, epithelial barrier). These macrophages clear bacteria by phagocytosis and secretion of metabolites, lysozyme, antimicrobial peptides, and proteases. When faced with overwhelming numbers of infectious agents, alveolar macrophages will produce and secrete an array of cytokines and chemokines, including IL-1, IL-6, TNF-α, and IL-8, which initiate the inflammatory response and recruit neutrophils to the alveolar space.[25]

Acute inflammation, mediated by granulocyte effector cells like neutrophils, is a necessary response to infection, irritation, or invasion in the lungs. It is the continuous initiation of the inflammatory response caused by stimulation with both fungi and bacteria, that leads to chronic inflammation and tissue injury. Under normal conditions, apoptosis will occur to contain neutrophilic influx before irreversible tissue damage can occur.[26] Opposite to controlled cell death is necrosis, which results in the release of damage-associated molecular patterns (DAMPs) into the extracellular space and a pro-inflammatory environment, leading to significant tissue damage in the host. Other mechanisms of cellular removal, like ETosis and efferocytosis, can stabilize inflammation and maintain tissue homeostasis.[27] It is the delicate balance between infection, inflammation and clearance that dictates disease progression and severity.

## CELLULAR COMPONENTS

### Mast Cells

Mast cells, first described in 1877 by Paul Ehrlich, were thought to be nourishment for other tissue-based cells because of their unique staining properties with alkaline aniline dye (e.g. toluidine blue or Alcian blue), in that the large granules are visible with this staining technique.[28] It wasn't until the 1950s that the connection between mast cells and allergy was made, in that mast cells were discovered to be the main source of histamine.[29-31] Mast cells are innate immune cells that develop from CD34+ hematopoietic precursor cells in bone marrow and circulate in the bloodstream in their immature form before migrating to tissue sites, where they eventually develop into the mature form.[32] Migration and maturation of mast cells is influenced mainly by stem cell factor (SCF) and its receptor, c-Kit (CD117). C-Kit is a transmembrane, tyrosine kinase receptor found on mast cells and is critical for chemotaxis, differentiation, survival, and activation of mast cells.[33] Mast cells are associated with allergic reactions due to the presence of the high-affinity receptor for IgE, FcεRI. Antigen/IgE-mediated activation of mast cells (as described above), is a complex process that eventually leads to degranulation and release of preformed granule contents, such as histamine, heparin, serotonin, proteases, tumor necrosis factor alpha (TNF-$\alpha$), as well as other immune mediators, cytokines, and chemokines.[34]

The role of mast cells in asthma has been shown in both allergic and non-allergic asthma. The preformed contents of mast cell granules have been found in the bronchoalveolar lavage (BAL) fluid of patients following allergen challenge.[35] Histamine causes bronchoconstriction and its levels in BAL have been correlated to airway hyperresponsiveness (AHR).[36,37] Mast cell-specific tryptase counteracts the effect that histamine has on smooth muscle contraction.[38] Tryptase also degrades the neuropeptide vasoactive intestinal peptide (VIP), which is a bronchodilator.[39] The release of preformed mediators of asthma is an early asthmatic response. In contrast, mast cells also play a role in "late" asthmatic responses in a variety of ways. Mast cells synthesize leukotriene $C_4$ ($LTC_4$) and prostaglandin $D_2$ ($PGD_2$), both of which are proinflammatory molecules. They are also capable of producing inflammatory cytokines, IL-4, IL-5, IL-13, and IL-1$\alpha/\beta$, which in turn recruit inflammatory cells like neutrophils, monocytes, basophils, eosinophils, and lymphocytes to the area.[35,40] An additional class of mediators produced by mast cells are growth factors, including SCF, granulocyte-macrophage colony-stimulating factor (GM-

CSF), and various other growth factors.[33] In addition to the classical Ig-E mediated degranulation of mast cells, degranulation can occur with physical changes (e.g. cold temperatures), chemical changes (e.g. osmolarity change), physical injury, inhalation of irritants, and viral infections.[35] Furthermore, IgG antibodies and various Fcγ receptors have been shown to play a role in mast cell degranulation.[41]

**Basophils**

Basophils are the rarest granulocyte found in circulation, constituting less than 1% of circulating leukocytes. Basophils are ~5 – 8μm in diameter and can be identified by staining with basic dyes (e.g. toluidine blue or Alcian blue). They have many features in common with mast cells, namely expression of the FcεRI and until the early 1990s were thought to be circulating mast cells. It is now known that basophils are an important source of $T_H2$ cytokines and therefore, play an important and unique role in allergy, as well as immune regulation.[42]

Recently, basophils have been found to be key mediators in $T_H2$ immunity via release of proteases (MCP-8 and MCP-11) that lead to increased vascular permeability and leukocyte infiltration to sites of inflammation. Basophils are also proposed as a source of IL-4, a cytokine that plays a principal role in driving $T_H2$ differentiation of naïve CD4 T cells. They also act as $T_H2$-oriented antigen-presenting cells (APCs) to facilitate $T_H2$ cell differentiation. This is done by basophils acquiring peptide antigen-MHC-II complexes from dendritic cells (DCs) via trogocytosis[42], a process in which the basophil extracts the surface molecule (in this case the antigen-MHC-II complex) from the DC it is conjugated to.[43]

Basophils can play roles in both IgE-dependent and IgE-independent allergic inflammation. The role of basophils in IgE-dependent allergic inflammation is through its high-affinity IgE receptor, FcεRI, and release of inflammatory mediators. The major preformed mediator stored in basophil granules is histamine, which increases blood vessel permeability and allows for entry of proinflammatory cells. Basophils are also capable of quickly producing leukotriene C4 (LTC4), which in turn breaks down into products LTD4 and LTE4, all three of these leukotrienes are bronchoconstrictors and increase vascular permeability. Activated basophils also express cytokines IL-4, IL-13, and GM-CSF, all of which are associated with a $T_H2$ immune response.[44] In an IgE-independent manner, mast cells release histamine after allergen exposure. The released histamine acts on the H4 receptor on basophils, causing them to migrate to tissues and release inflammatory mediators. However, the mechanisms of the IgE-independent

inflammatory pathway remain ill-defined. The role of basophils in allergy and asthma is an area that is not thoroughly understood, due to the scarcity of basophils, lack of relevant cell lines, and inability to use biochemical tools to identify and purify basophil cell populations.[45]

**Neutrophils**

Neutrophils are the most predominant leukocyte in circulation, comprising 55 – 70% of the total population. They circulate as spheres ~12-15µm in diameter and under microscopy, exhibit a characteristic multilobed, segmented nucleus.[46] Neutrophils are produced in the bone marrow and emerge as mature neutrophils via a process termed myelopoiesis. Myelopoiesis begins with the differentiation of pluripotent stem cells into myeloid progenitors, common myeloid progenitors (CMPs) and granulocyte-monocyte progenitors (GMPs), which then develop six different subtypes of neutrophils: the myeloblast; the promyelocyte, in which primary, or azurophilic granules appear; the myelocyte, where cell division stops and secondary, or specific granules develop; the metamyelocyte, in which tertiary granules appear; band cells; and finally, mature neutrophils, characterized by their multilobed nucleus and cytoplasm containing granules.[47] The differentiation of neutrophils is controlled by a number of grown factors, including SCF, IL-3, IL-6, GM-CSF, and granulocyte-colony stimulating factor (G-CSF).[48]

Neutrophils are the first circulating cell to reach the infection site, where they will contain and eliminate foreign invaders by either phagocytizing any opsonized microorganism, releasing reactive oxygen intermediates (ROIs), releasing cytotoxic granules, or formation of neutrophilic extracellular traps (NETs). NETs are formed when neutrophils eject their nuclear chromatin and bactericidal proteins to entrap and kill invading microorganisms. This process of NET formation is termed NETosis.[49] Neutrophils contain at least four different types of granules in the cytoplasm. Primary, or azurophilic granules are lysosomes that are the main storage site of the most toxic mediators, including elastase, myeloperoxidase, cathepsins, and defensins. Secondary, or specific granules, are the most abundant and contain enzymes such as lysozyme, collagenase, and elastase. These granules stain neutral (i.e. neither basic nor acidic) which distinguishes their granules from that of basophils or eosinophils.[47,50] Tertiary granules contain matrix metalloprotease 9 (i.e. gelatinase B), belonging to a family of enzymes that breakdown the extracellular matrix (ECM).[47,50,51]

After clearance of foreign particles, neutrophils die through one of three mechanisms: NETosis, apoptosis (programmed cell death), or necrosis (uncontrolled cell death). Dysregulation of neutrophil production and/or death often results in disease.[47] Uncontrolled asthma has been linked to an increase number of neutrophils in airways, where they are a source for proinflammatory cytokines IL-1, IL-3, IL-6, IL-8, IL-12, IFN-γ, GM-CSF, and MIP (macrophage inflammatory protein). Increased numbers of neutrophils and cytokines, IL-8, and IL-17, both strong neutrophilic chemoattractants, are the hallmarks of neutrophilic asthma. Additionally, throughout the course of neutrophilic asthma, neutrophils release IL-8, thus recruiting more neutrophils to the airway, resulting in a positive feedback loop. The number of neutrophils and the concentration of IL-8 in BAL allow neutrophilic asthma to be distinguished from other forms of asthma. Recent studies have shown that neutrophils are not only closely related to disease severity, but also initiation of allergic inflammation and allergic sensitization.[52,53]

**Mononuclear Phagocytes**

Mononuclear phagocytes include circulating monocytes and tissue residing macrophages. Monocytes circulate in the blood as precursors to macrophages, are ~10 – 15µm in diameter, have a distinct bean-shaped nucleus, and a finely granular cytoplasm. Monocytes that are called classical monocytes are $CD14^{++}CD16^{-}$ and are rapidly recruited to injury sites, where they release inflammatory mediators. Non-classical monocytes are $CD14^{+}CD16^{+}$ contribute to tissue repair after injury by 'crawling' along endothelial surfaces. Once monocytes migrate into tissues, they mature into macrophages, whose main purpose is to ingest and kill microorganisms mainly via generation of reactive oxygen and nitrogen species, as well as proteolytic digestion.[54]

Macrophages also play a role in the immune system by ingesting dead cells before their mediators can be released. Macrophages secrete different cytokines, like TNF, IL-1, and IL-6, that enhance leukocyte recruitment from the blood to tissues. Macrophages can also play a role in the adaptive immune system, as they can serve as APCs to display antigens that activate T cells during the effector phase of a T cell-mediated immune response.[55]

Maturation of macrophages can happen in one of two ways: (1) Tissue residing macrophages differentiate in specific organs such as the brain, liver, lungs, or spleen and become specialized microglial cells, Kupffer cells, alveolar macrophages, or sinusoidal macrophages, respectively. These specialized

cells are derived from precursor cells present in the yolk sac and liver of a fetus. (2) In the bone marrow, monocytes develop from hematopoietic stem cells (HSC) to myeloid lineage precursor cells, circulate in the blood, and migrate to tissues where they mature into macrophages.[50] Lung macrophages can be divided into two distinct types: alveolar macrophages (AMs) and interstitial macrophages (IMs). AMs reside in the inner surface of the lung, whereas IMs reside in the interstitial space. AMs can be further divided by mode of activation. Classically activated macrophages (M1 cells) can be activated by IFN-γ and lipopolysaccharide (LPS), inducing a nonallergic immune response by releasing TNF-α and IL-1β, skewing the immune response to a $T_H1$ response. Alternatively activated macrophages (M2a cells) can be activated by IL-4, IL-13, and IL-33, inducing an allergic immune response by releasing IL-4 and IL-13, hallmark $T_H2$ cytokines.[56]

**Eosinophils**

Eosinophils are a granular leukocyte found in circulation that are ~12 – 17μm in diameter. In healthy individuals, they represent ~1 – 3% of circulating leukocytes. The hallmark feature of eosinophils under light microscopy are the dense cytoplasmic granules that stain red with acidophilic dyes, such as eosin.[57] These large granules, known as secondary granules, are what distinguish eosinophils from neutrophils and basophils. They contain proteins, cytokines, chemokines, growth factors, and enzymes that are known mediators of inflammation.[58] The prevalent proteins present in the granules include major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), and eosinophil-derived neurotoxin (EDN).[59] MBP functions by disrupting the lipid bilayer on the cell membrane and functions as a mediator of the inflammatory response. It is known to stimulate histamine release from basophils and mast cells, activate neutrophils and platelets, and enhances superoxide generation by alveolar macrophages.[60] ECP is a ribonuclease that is used as a marker for eosinophilic disease. Its cellular effect is not due to ribonuclease activity, but rather its ability to induce cellular apoptosis via caspase-8 activation.[61] EPO is a toxic ROS that disrupts cell walls and can post-translationally modify proteins at the amino acid level.[62] EDN functions by activating dendritic cells involved in the expression of inflammatory cytokines, chemokines, growth factors, and receptors.[60]

Eosinophils develop, in ~one week, from bone marrow pluripotent progenitors in response to differentiating cytokines, IL-5, IL-3, and GM-CSF. After maturation, eosinophils enter the peripheral blood

and migrate to tissues via cytokines produced by $T_H2$ cells, IL-5 and eotaxin (CCL-11). Additionally, recruitment of eosinophils to infection sites is facilitated by adhesion molecules, VCAM-1 and E-selectin.[50] It is here, in the tissues, that eosinophil preform their primary function – degranulation and release of mediators. [63,64]

**Dendritic Cells**

Dendritic cells (DCs) are cells that act as a 'bridge' between the innate and adaptive immune systems. They are specialized APCs responsible for initiation of the adaptive immune system by capturing foreign antigens present outside of the cell, moving to lymph nodes, and presenting the antigens to naïve T cells.[50] DCs are one of the first immune cells to come into contact with allergens. Normally located on the basolateral side of epithelial cells in the lung, they sample antigens on the lumen side by extending dendrites across the epithelial barrier. They are able to do this while still maintaining the protective barrier provided by the epithelial layer by forming tight junctions with epithelial cells.[65] By presenting captured antigen to naïve T cells, DCs can play a role in directing $T_H$ differentiation and in the case of allergic asthma, skewing differentiation to a $T_H2$ response. Numerous rodent models have data to support that DCs play a critical role in the development and maintenance of allergen-induced airway inflammation and AHR.[66]

## ALLERGIC ASTHMA

### Allergy and Hypersensitivity

According to the World Allergy Organization, allergy is defined as “a hypersensitivity reaction initiated by immunological mechanisms”. Commonly, asthma is categorized as a type I hypersensitivity with the hallmark immunoglobulin, IgE, present in most patients. However, this typical definition of does not explain all clinical observations associated with asthmatic patients.[67] Hypersensitivity reactions can be either cell or antibody mediated.[68] The antigens that elicit an immunological response are termed allergens. Hypersensitivities can be categorized into different categories based on mechanism and time for the reaction to occur. This categorization was initially defined by Gell and Coombs in 1963 into four categories[69] and subsequent revisions and additions have been made to their classification scheme.

Type I, or immediate hypersensitivity, is IgE antibody- mediated and occurs within 2-30 minutes after antigen exposure, this is the prototypical allergic reaction. IgE antibodies are produced in response to allergens and then bind to mast cells or basophils. The release of preformed inflammatory mediators, such as histamine from basophils or mast cells via degranulation, is caused by antigen induced crosslinking of Ig-E bound to cell surface FcεRI. Examples of Type I hypersensitivity include, allergic asthma, allergic rhinitis, systemic anaphylaxis, and urticaria.[70,71]

Type II, or cytotoxic hypersensitivity, involves the destruction of cells through cytotoxic mechanisms and occurs minutes to hours after antigen exposure, with peak response at ~1 day.[70] Antibodies (IgG or IgM) specific to an antigen on the cell surface bind, initiating the cytotoxic mechanism. Cell destruction can be mediated by complement or by phagocytes containing the Fc antibody receptors on their cell surface. Activation of complement results in loss of cell membrane integrity and eventually, cell death. Alternatively, phagocytosis of an antibody-coated target cell by neutrophils, monocytes, or macrophages via surface IgG Fc receptors, a process known as opsonization. Additionally, antibody-dependent cellular cytotoxicity can destroy target cells by the non-phagocytic action of T cells or natural killer cells (NKC). Direct contact between the target and effector cells is necessary.[72] Examples include hemolytic anemia, erythroblastosis fetalis, and thrombocytopenia.[70]

Type III, or immune complex hypersensitivity, are caused by antigen-antibody complexes that are formed in the blood, moving to tissues. Immune complexes are produced in all antibody responses but

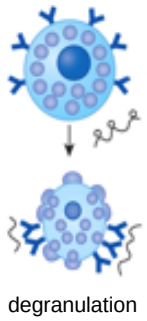
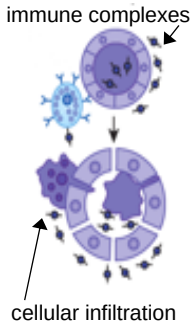
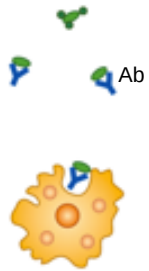
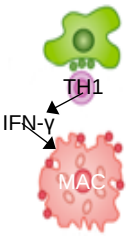
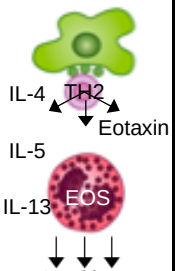
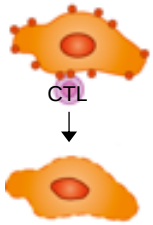
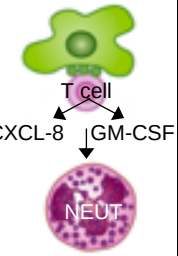
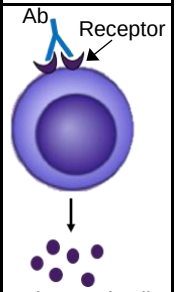
the ability to cause disease is determined by size, number, affinity, and type. Large complex aggregates will bind serum complement and will be cleared phagocytes. However, smaller complexes that are formed when an excess of antigen is present, will accumulate in the walls of blood vessels. It is here that these complexes will attach to the Fc receptor on leukocytes to induce complement activation leading to mast cell degranulation, leukocyte chemotaxis, or inflammation.[72,73]

Type IV hypersensitivities are delayed reactions (occurring 48-72 hours after antigen exposure) mediated by antigen-specific T cells ($CD4^+$ or $CD8^+$) and can be further broken down into subclassifications.[70] Type IVa hypersensitivity is associated with T-helper 1 ($T_H1$) cells activating macrophages by secreting interferon-gamma (IFN-$\gamma$). The activated macrophages drive the production of complement fixing antibody isotypes (IgG1, IgG3), secrete proinflammatory cytokines (TNF, IL-12) and chemokines, and enhance CD8+T cell responses. The tuberculin reaction is an example of a Type IVa reaction. Type IVb is associated with $T_H2$ type immune responses. $T_H2$ cells secrete IL-4, which stimulates B-cell production of IgE that can lead to mast cell degranulation or IgG antibody production. $T_H2$ cells also secrete IL-5 and IL-13, which lead to eosinophil activation and macrophage activation, respectively. In IVc hypersensitivity reaction, T cells can also act as effector cells. T-cells migrate to the tissue and kill tissue cells in a perforin/granzyme B, creating pores in the cellular membrane and release of serine proteases[74] or causes apoptosis in a FasL-dependent manner.[75] Type IVc and IVa reactions often occur together and are commonly seen in maculopapular or bullous skin disease.[76]

Type IVd involve the production of CXCL-8 and GM-CSF by T-cells which recruit and maintain a neutrophilic environment. Examples of Type IVd hypersensitivity is acute-generalized exanthematous pustulosis (AGEP) and Behcet's disease.[77]

More recently Type V hypersensitivity has been added to Gell and Coombs' classification. Type V occurs when an antibody binds to a cell's receptor instead of its surface, resulting in abnormal cell signaling. The strongest example of this type of hypersensitivity is Graves' disease.[78] Table 1 is included as a summary of the different types of sensitivities.[72]

**Table 2. Types of Hypersensitivities**

| | Type I | Type II | Type III | Type IVa | Type IVb | Type IVc | Type IVd | Type V |
|---|---|---|---|---|---|---|---|---|
| **Descriptive Name** | Immediate or anaphylactic | Cytotoxic, Antibody-dependent cytotoxicity | Immune complex hypersensitivity | Cell-mediated | | | | Autoimmune |
| **Response Time** | 2 – 30 Minutes | Minutes to Hours | 3 – 8 Hours | 2 – 3 days | | | | Minutes to Hours |
| **Immune Reactant** | IgE | IgM, IgG | IgG, IgM | TH1 Cells, IFN-γ, TNF-α | TH2 Cells IL-4, IL-5, IL-13, Eotaxin | CTL, Perforin, Granzyme B, FasL | T-Cells, CXCL-8, GM-CSF | IgM, IgG, Complement |
| **Antigen** | Soluble | Cell- or Matrix-Associated | Soluble Complexes | Soluble | Soluble | Cell-Associated | Soluble | Cell Surface Receptor |
| **Effector** | Basophils, Mast Cells | FcR+ Cells | Complement | Monocytes | Eosinophils | T-Cells | Neutrophils | Cell Surface Receptors |
| **Effector Mechanism** |  degranulation |  immune complexes; cellular infiltration |  Ab; phagocytosis |  TH1; IFN-γ; MAC; Chemokines, Complement |  IL-4; TH2; Eotaxin; IL-5; IL-13; EOS; Cytokines, Chemokines |  CTL; Cell death, Apoptosis |  T cell; CXCL-8; GM-CSF; NEUT; Neutrophil activation and recruitment |  Ab; Receptor; Abnormal cell function |
| **Example** | Allergic asthma, Systemic anaphylaxis | Hemolytic Anemia, Some drug allergies | Serum sickness, Farmer's Lung | Tuberculin reaction, Contact dermatitis | Chronic asthma, Chronic allergic rhinitis | Contact dermatitis, Hepatitis | Behcet's Disease | Graves' Disease, Myasthenia gravis |

[a] Adapted from Pharmacotherapy: A Pathophysiological Approach by Sylvia, LM, 2014, New York, NY: ©Mc-Graw Hill.[72]

**Asthma**

According to the National Heart, Lung, and Blood Institute (NHLBI) at the National Institutes of Health (NIH), asthma is a “chronic (long-term) lung disease that inflames and narrows the airways,” causing “recurring periods of wheezing (a whistling sound when you breathe), chest tightness, shortness of breath, and coughing”.[79] The hallmarks of asthma are recurrent and “reversible airway obstruction, chronic bronchial inflammation with eosinophils, bronchial smooth muscle cell hypertrophy and hyperreactivity, and increased mucus secretion”.[80] Globally, an estimated 300 million people suffer from asthma, with predictions that an additional 100 million people will be diagnosed by the year 2025.[79] According to the Centers for Disease Control (CDC)’s National Health Interview Survey conducted in 2015, approximately 25 million Americans have asthma, equating to an economic burden of $56 billion in the US.[81]

The cause of asthma is unknown, however, the interaction between genetic and environmental factors has been the focus of much research in determining the cause. Triggers of asthma can be extrinsic: environmental allergens (e.g. pollen, dust mites, pet dander, molds), smoke, pollution, or chemicals; or intrinsic: respiratory infection, exercise, or changes in weather.[82] Asthma is commonly categorized based on these triggers, allergic (extrinsic), non-allergic (intrinsic), or a combination of both. Asthma can also be classified as atopic, in which there is evidence of allergen sensitization or nonatopic, meaning no clear evidence of allergen sensitization. In either type, episodes of bronchospasm can be triggered by extrinsic or intrinsic stimuli or a combination of both. Many inflammatory cells play a role in asthma; in particular, eosinophils, mast cells, macrophages, lymphocytes, neutrophils, and epithelial cells. There is emerging evidence for differing patterns of inflammation: highly eosinophilic, highly neutrophilic, mixed inflammatory, and pauci-granulocytic (normal levels of both eosinophils and neutrophils). These subgroups differ in etiology, pathology, and response to treatment. [83]

Allergic asthma is the most common form for asthma, accounting for more than half of asthma sufferers, and is triggered by the inhalation of allergens. It is often associated with an overreaction of the $T_H2$ response to allergens. Allergic asthma is the prototypic $T_H2$ mediated disease and the typical sequence of events consist of exposure to allergen, release of thymic stromal lymphopoietin (TSLP),

activation of T cells and B cell class switching to produce IgE, binding of IgE to FcεRI on mast cells, reintroduction of allergen, activation of mast cells, and secretion of mediators leading to the associated pathological reactions (Figure 3).[50]

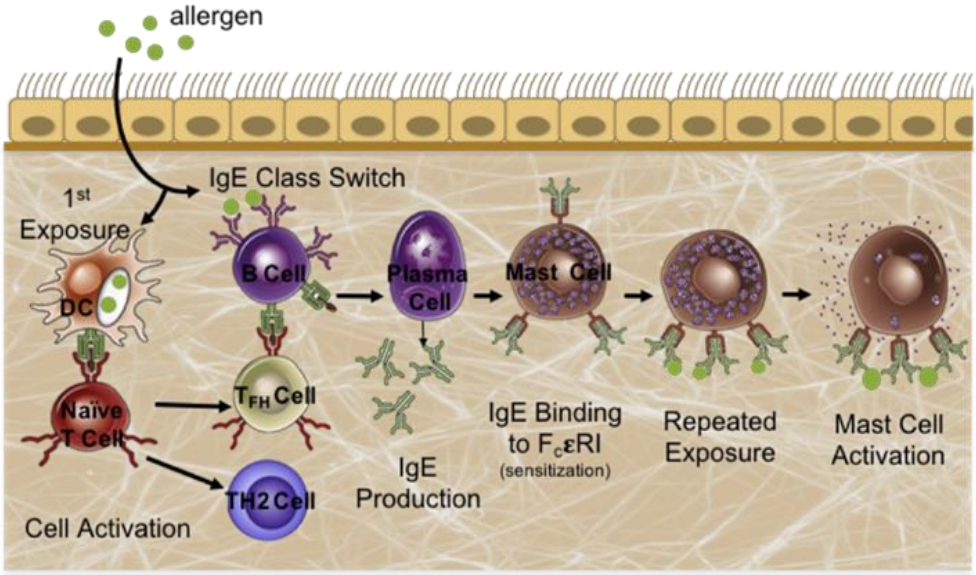



**Figure 3. Sequence of Events in a Typical TH2-mediated Allergic Reaction.** This cascade is initiated by exposure to the allergen, stimulating a class switch by B cells to produce IgE. IgE sensitizes mast cells by binding to the FcεRI and repeat exposure to the allergen activates mast cells, resulting in secretion of mediators into the surrounding tissue. Adapted from Cellular and Molecular Immunology by Abbas, A. K., A. H. Lichtman and S. Pillai, 2015, Philadelphia, PA: © Elsevier Saunders.[50]

Cytokines produced by $T_H2$ cells are responsible for most of the features of asthma; IL-4 stimulates IgE production, IL-5 activates eosinophils, and IL-13 stimulates mucus production and promotes IgE production by B cells. IgE coats the surface of mast cells, which, on exposure to allergen, release their granular contents: histamine, cysteinyl leukotrienes, and prostaglandins.[84] This stimulates two waves of reaction: an early (immediate) phase and a late phase. The early reaction is dominated by bronchoconstriction, increased mucus production and vasodilation. The late-phase reaction is primarily inflammatory, with activation of eosinophils, neutrophils, and T cells. Additionally, epithelial cells are activated to produce TSLPs and chemokines (e.g. eotaxin) that promote recruitment of more $T_H2$ cells and eosinophils, as well as cytokines, IL-25 and Il-33, causing recruitment of type 2 innate lymphoid cells (ILC2) and amplification of the inflammatory reaction (Figure 4).[84] Chronic bouts of inflammation lead to structural changes in the bronchial wall, referred to as airway remodeling. These changes include

hypertrophy of bronchial smooth muscle and mucus glands, increased vascularity, and deposition of subepithelial collagen. These changes may occur several years before symptoms appear.[80]

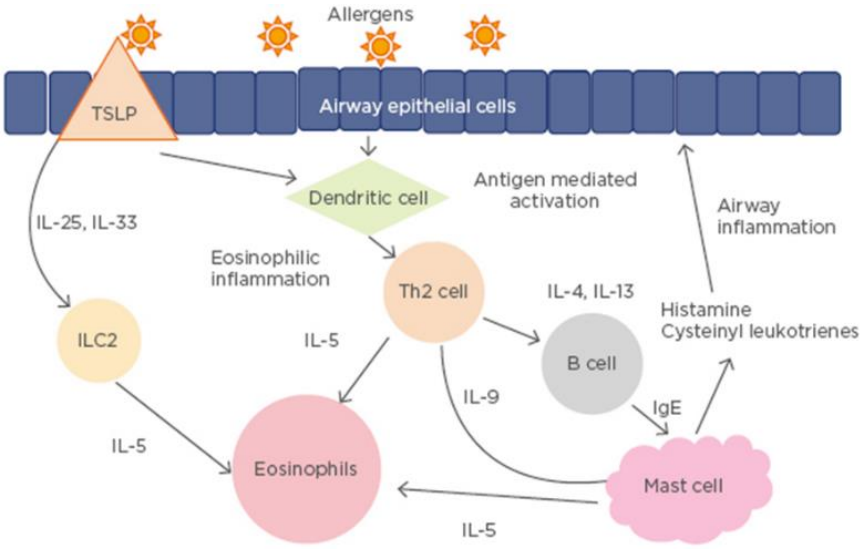



**Figure 4. $T_H2$-mediated Pathogenesis of Asthma.** Asthma pathophysiology starts with the release of TSLP by epithelial cells after contact with allergens. This activates $T_H2$ cells produce IL-4, IL-5, and IL-13 leading to IgE formation and eosinophil activation. Mast cells are activated once IgE is crosslinked to its' high affinity IgE receptors leading to release of granular mediators, leading to airway inflammation. ILC2s also play a role by production of IL-5 leading to eosinophil activation. From "Asthma: Diagnosis and Treatment" by So, J. Y., A. J. Mamary and K. Shenoy, 2018, European Medical Journal 3, (4), p. 115. CC BY-NC 4.0[84]

***Diagnosis.*** Asthma can be diagnosed in a clinical setting, however, there is no standard test available due to the variability in disease progression and presentation. A thorough personal history, physical exam, and spirometry are needed for diagnosing asthma. Coughing, wheezing, tightness in chest, or shortness of breath are all physical symptoms that may be indicative of asthma. Differential diagnosis is important to rule out other diseases that may present as asthma, these can include chronic obstructive pulmonary disease (COPD), vocal cord dysfunction, cardiovascular diseases, or anxiety.[84] Spirometry is a lung function test that is recommended to confirm an asthma diagnosis and requires breathing into a spirometer that will record breathing patterns. The key measurements made during testing are forced vital capacity (FVC), which is a measure of the maximum amount of air that is forcefully exhaled after deepest inhalation, and forced expiratory volume ($FEV_1$), which is a measure of the volume of air that can be forced out in one second.[85] If FVC is lower than normal, something is obstructing the ability to breathe. $FEV_1$ is used to calculate the ratio, $FEV_1$/FVC, that represents percentage of lung

capacity that is exhaled in one second. A higher ratio would be indicative of healthy lungs, with a lower ratio suggesting airway blockage.[86,87]

***Treatment.*** There is currently no cure for asthma. The primary, long-term goal for asthma management is to control symptoms, maintain normal physical activities, and reduce exacerbations, airway obstruction, and therapeutic side effects. Current asthma control is a combination of pharmacological and nonpharmacological therapy. This model has been shown to improve treatment success and is based on a cycle of assess, adjust, and review (Figure 5).[88]

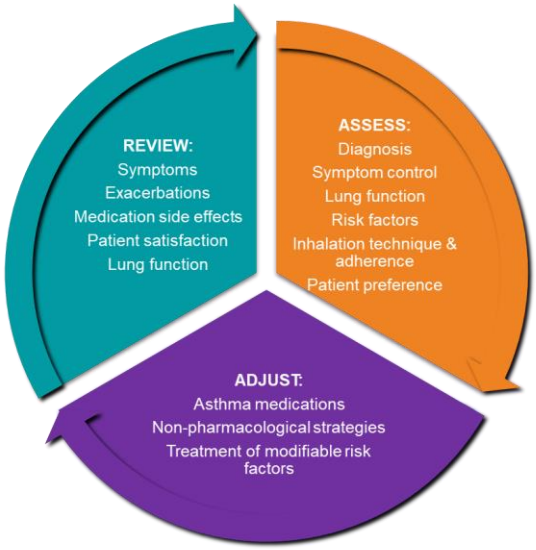



**Figure 5. Cycle of Combination Asthma Treatment.** Adapted from "Diagnosis and management of asthma - Statement on the 2015 GINA Guidelines" by Horak, F., et al., 2016, Wien Klin Wochenschr 128(15-16): 544**.** CC BY 4.0

The current gold standard for treatment of asthma is inhalation therapy. Inhalers are an ideal choice because it leads to higher concentrations of drug in the lungs, fewer systemic side effects, and higher adherence to treatment protocol. There are three distinct categories for long-term treatment. Controllers are taken regularly to minimize inflammations, exacerbations, and symptoms. Relievers are taken as needed to reduced asthmatic exacerbations. The goal is to minimize the need for relievers. Add-on treatment is used in severe cases when symptoms persist despite high-dose combination therapy. Table 3 is included as a reference for the three different types of treatment.[84]

**Table 3. Pharmaceutical Categories for Asthma Treatment**

| Controller | Reliever | Add-on Therapy |
|---|---|---|
| Inhaled corticosteroid (ICS) | Short-acting beta-2-agonists (SABA) | Anti-IgE therapy |
| ICS/LABA (long-acting beta-2-agonist) combination | Short-acting anticholinergics (LAMA) | Systemic/oral corticosteroids (OCS) |
| Leukotriene receptor agonists (LTRA) | | Anti-IL5 therapy |
| Long-acting anticholinergics (LAMA) | | Special (phenotype-specific) treatments and interventions by specialized centers |

[a]Adapted from "Asthma: Diagnosis and Treatment" by So, J. Y., A. J. Mamary and K. Shenoy, 2018, European Medical Journal 3, (4), p. 118. CC BY-NC 4.084.[84]

Beta-2 agonists are bronchodilators that help control acute asthmatic exacerbations. These function by binding to beta-2 adrenergic receptors on bronchial smooth muscle cells, causing smooth muscle relaxation and bronchodilation. Short-acting beta-2 agonists (SABAs) are used for mild asthma and acute exacerbations. SABAs work quickly, within 1 – 5 minutes and a peak effective time of two hours. Long-acting beta-2 agonists (LABAs) have bronchodilatory effects lasting up to 12 hours. LABAs should be prescribed with inhaled corticosteroid (ICS) treatment to minimize asthma-related death or life-threatening events. LABA/ICS combination therapy has proven to be safe and effective, with lower asthma exacerbations and improved lung function.[84]

Corticosteroid, or ICS, treatment is of importance when treating patients with persistent asthma, especially those with an eosinophilic phenotype. These drugs downregulate eosinophil and mast cell activation, leading to a decrease in airway hyperresponsiveness and inflammation. Short-term systemic corticosteroids use can help in decreasing systemic inflammation and bronchoconstriction, however, this is not a long-term solution due to the long-term systemic side effects associated with use.[89]

Leukotrienes are lipid mediators that play a pivotal role in bronchoconstriction and airway inflammation associated with asthma. Leukotriene-modifying drugs can work by either blocking synthesis of the leukotriene or being a competitor for leukotriene receptors. Leukotriene receptor agonists have shown to improve asthmatic symptoms and show promise in preventing or reversing airway structural changes.[90]

Long-acting muscarinic antagonists (LAMAs) have been used for years to relieve bronchoconstriction and control severe asthma. Acetylcholine binds to muscarinic receptors, as part of the parasympathetic system, leading to bronchoconstriction, increased mucus secretion, inflammation, and airway remodeling. LAMAs function by disrupting muscarinic receptor activation, leading to bronchodilation.[91,92]

Add-on therapies, include biological therapies, are used to treat severe asthmatics and careful consideration should be taken before starting. The majority of biological therapies are monoclonal antibodies against immunoglobulins, cytokines, or their receptors, including IgE, IL-4, IL-5, or TSLP. These have proven to be very effective in the long-term treatment of previously uncontrolled, severe asthmatics.

## HYPERSENSITIVITY PNEUMONITIS

Hypersensitivity pneumonitis (HP), also termed extrinsic allergic alveolitis, is a complex respiratory syndrome involving inflammation of the lung parenchyma (specifically the alveoli, terminal bronchiole, and alveolar interstitium). This inflammation is due to a delayed allergic reaction in response to inhaled allergens (Table 4).[93] This reaction is secondary to repeated, prolonged inhalation of substances to which a person has been sensitized and is hyperresponsive to.[94]

**Table 4. Common Types of Hypersensitivity Pneumonitis Antigens**

| Class of Antigens | Specific Antigens | Sources | Type of Disease |
|---|---|---|---|
| Bacteria | *Saccharopolyspora rectivirgula*<br>*Thermoactinomyces vulgaris* | Moldy hay, grain | Farmer's lung |
| Fungi, Yeast | *Aspergillus* species<br>*Trichosporon cutaneum*<br>*Penicillium* species<br>*Penicillium casei*<br>*Alternaria* species | Moldy hay, grain<br>Moldy compost and mushrooms<br>Contaminated houses<br>Moldy cork<br>Moldy cheese or cheese casings<br>Contaminated wood pulp or dust | Farmer's lung<br>Mushroom worker's lung<br>Japanese summer-type HP<br>Suberosis<br>Cheese washer's lung<br>Woodworker's lung |
| Mycobacteria | *Mycobacterium avium - intracellulare* | Mold on ceiling, tub water<br>Mist from pool water, sprays, and fountains | Hot tub lung<br>Swimming pool lung |
| Animal Proteins | Proteins in avian droppings, serum, and on feathers<br>Avian proteins<br>Silkworm proteins | Parakeets, budgerigars, pigeons, parrots, cockatiels, ducks<br>Feather beds, pillows, duvets<br>Dust from silkworm larvae and cocoons | Pigeon breeder's lung, bird fancier's lung<br>Feather duvet lung<br>Silk production HP |
| Chemicals | Diisocyanates, trimellitic anhydride | Polyurethane foams, spray paints, dyes, glues | Chemical worker's lung |

[a]Adapted from Spagnolo, P., et al. (2015). Hypersensitivity Pneumonitis: A Comprehensive Review. ©Esmon Publicidad [93]

The prevalence of this disease is hard to evaluate as the syndrome itself is highly variable in not only severity of symptoms, but also clinical presentation and prognosis. Other variables in this syndrome include differences in causative agent, exposure duration, and host response to the antigen.[95] The complexity of the disease is also evident in the fact that not all individuals exposed to the same causative factors will develop HP nor will those with the disease always be symptomatic.[96]

HP has historically been separated into three categories: acute, subacute, and chronic. However, there is some debate as to the usefulness of having three forms and a new classification to two forms, acute and chronic, has been proposed due to the difficulty in differentiating acute and subacute forms.[97] In the acute phase, symptoms include fever, chills, cough, fatigue, and muscle and joint pain. Symptoms appear four to eight hours after exposure and last for a few hours to a few days. The subacute form appears with the same symptoms but happens gradually, over a few days to a few weeks. Symptoms of chronic HP include cough, difficulty breathing, fatigue, and weight loss, occurring over several months to years. This classification system is imperfect, but the overall grouping with two distinct groups, acute/subacute and chronic HP being common among the scientific community (Table 5).[97,98]

**Table 5. Classification of Hypersensitivity Pneumonitis Types**

| Type of HP | Symptom Onset | Symptoms | High-resolution Computed Tomography (HRCT) Findings | Histological Findings |
|---|---|---|---|---|
| Acute/Subacute | hours to weeks | fevers, chills, cough, fatigue, and muscle and joint pain | small, indistinct, centrilobular nodules (tissue masses), evidence of air trapping during exhalation, and ground glass opacities (GGOs) | interstitial infiltrate composed of lymphocytes and plasma cells, cellular bronchiolitis, and formation of granulomas, usually with cholesterol clefts |
| Chronic | months to years | cough, difficulty breathing, fatigue, and weight loss | reticular patterns, honeycombing, and traction bronchiectasis (i.e. irreversible dilatation of bronchi and bronchioles) in the upper and middle lobes | fibrosis |

[a]Adapted from Vasakova, M., F. Morell, S. Walsh, K. Leslie, and G. Raghu (2017). American Journal of Respiratory and Critical Care Medicine 196(6): 680-689[97] and Soumagne, T. and J. C. Dalphin (2018). Expert Rev Respir Med 12(6): 493-507. ©Taylor & Francis Group [98]

**Farmer's Lung Disease**

Farmer's lung disease (FLD), a common type of hypersensitivity pneumonitis, is an immunologically mediated inflammatory disease of the lung. It is associated with intense or repeated exposure to inhaled biological dusts. The classic presentation of farmer's lung is from inhalational exposure to thermophilic actinomyces and various fungi.[99] A causative microorganism associated with farmer's lung is *Saccharopolyspora rectivirgula,* a Gram positive thermophilic bacteria that is commonly found in moldy hay.[100]

The pathogenesis of FLD is complex and not fully understood, with components of both type III and IV hypersensitivity reactions contributing to disease progressiong.[102] Following sensitization with *S. rectivirgula*, interaction between immune cells – Ag, cell – cell, and cell – mediators occurs leading to the accumulation of inflammatory cells in the lungs.[103] In a type III reaction, soluble *S. rectivirgula* antigens bind with circulating IgG antibodies to form an excess of small, insoluble immune complexes that are not phagocytosed. These immune complexes accumulate and deposit in tissues and blood vessel walls. Once deposited, they initiate an acute inflammatory response via activation of the classical complement pathway, leading to recruitment of leukocytes (e.g. neutrophils), and release of enzymes and toxic molecules (Figure 6).[101] Initiation of this pathway begins with the immune complexes binding to complement protein, C1, resulting in the production of proteolytic fragments: C3a, C4a, C5a, which causes recruitment and activation of inflammatory cells (e.g. mast cells, macrophages, neutrophil). Another product generated by the complement cascade, C3b, acts as an opsonin on the immune complexes to activate phagocytes and leads to additional damage to the vessel walls.[104]

In a type IV reaction, both cytokine-mediated inflammation and T cell mediated cytotoxicity result in tissue damage (Figure 7). In cytokine-mediated inflammation, CD4+ T cells will release cytokines that recruit and activate leukocytes. For example, $T_H17$ cells will produce IL-17 which recruits neutrophils or $T_H1$ cells produce IFN-γ and TNF which activate macrophages and other leukocytes. Tissue damage is a result of mediators produced by these leukocytes, such as ROS, lysosomal enzymes, and proinflammatory cytokines.[101] T cell-mediated cytolysis occurs when CD8+ T cells recognize specific peptide – MHC complexes on a target cell to induce apoptosis. The mechanism in which this occurs is via

the release of granules, perforin and granzymes, which form pores in the target cell membrane and act as serine protease, respectively.[105]

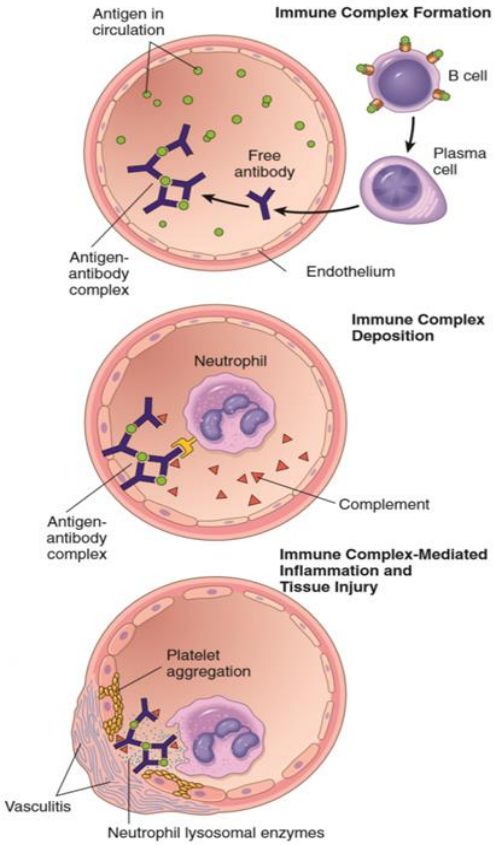



**Figure 6. Immune Complex Formation in Type III Hypersensitivity.** Immune complexes are formed when multiple antigens bind antibodies (IgG). Once deposited, complement cascade is initiated, leading to neutrophil recruitment, release of enzymes and toxic molecules, resulting in tissue damage. • From Robbins Basic Pathology by Kumar, V., A. K. Abbas, J. C. Aster and J. A. Perkins, 2017, Philadelphia, PA: ©Elsevier Saunders.[101]

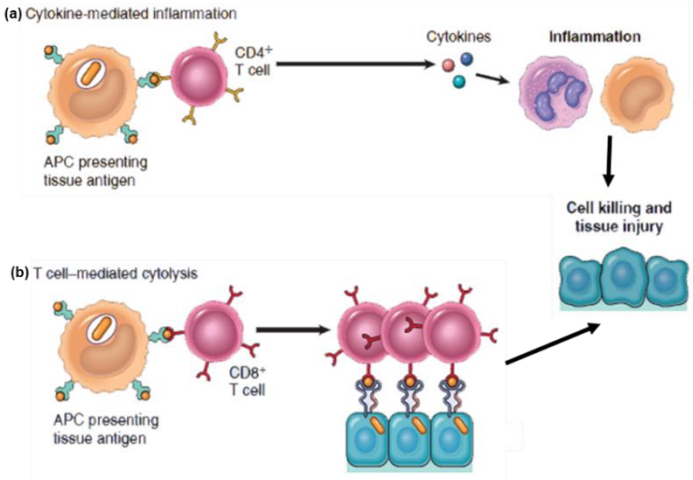



**Figure 7. Mechanism of Type IV Hypersensitivity.** (a) In cytokine-mediated inflammation, CD4+ T cells recruit and activate inflammatory cells. (b) In T cell-mediated cytolysis, CD8+ cytotoxic T lymphocytes cause apoptosis of cells. Both mechanisms result in tissue injury. From Robbins Basic Pathology by Kumar, V., A. K. Abbas, J. C. Aster and J. A. Perkins, 2017, Philadelphia, PA: ©Elsevier Saunders.[101]

***Diagnosis.*** Currently, there is not a consensus in diagnosis of farmer's lung. Diagnosing requires a combination of differential diagnosis, medical history, identification of causative agents, physical exam, screening of serum and BAL for IgGs specific for *S. rectivirgula*, high-resolution computed tomography (HRCT) of chest and lung biopsy.[106]

Acute HP can present as common diseases, such as colds and influenza, as symptoms include fever, cough, and difficulty breathing. Chronic HP can be misdiagnosed as other lung diseases like

idiopathic pulmonary fibrosis (IPF), pneumonia, or lung cancer. Implementing differential diagnosis to rule out other diseases is a crucial step in correct diagnosis of HP.[107]

Once suspicion of HP is raised, an in-depth patient history, including evidence of respiratory dysfunction and extensive investigation into environmental and occupational exposure s to identify relevant agents and gather a timeline of exposure to onset of symptoms. Physical examination and general laboratory tests cannot be used as standalone tests in diagnosing HP as many symptoms and elevated immunoglobulins are indicative of many other diseases. Using precipitin tests that are specific to *S. rectivirgula* can aid in diagnosis but cannot be used for all patients for various reasons like low sensitivity to antigens, poor patient sampling, or lack of knowledge about causative agents.

HRCT can be a useful tool in diagnosis of HP due higher sensitivity and specificity compared to plain chest X-ray. Several findings on HRCT are common in patients diagnosed with HP, including small, indistinct, centrilobular nodules (tissue masses), evidence of air trapping during exhalation, and ground glass opacities (GGOs). GGOs are nonspecific and viewed as hazy opacity on HRCTs; they may be indicative of partial filling of air spaces by fluids, thickening of the interstitium, or collapse of alveoli. Reticular patterns, honeycombing, and traction bronchiectasis (i.e. irreversible dilatation of bronchi and bronchioles) in the upper and middle lobes are findings associated with chronic forms of HP. These finding are also found in IPF; however, distribution is commonly in the lower lungs instead of the upper and middle.[108]

Histological findings on lung biopsies are usually not needed in diagnosis of HP, but in cases where biopsy was conducted, abnormalities were seen in patient samples. In acute/subacute HP, fibrin deposition, neutrophilic influx, and formation of small granulomas. In 75% of cases, a “histological triad” is present, consisting of interstitial infiltrate composed of lymphocytes and plasma cells, cellular bronchiolitis, and formation of granulomas, usually with cholesterol clefts. Histologically, chronic HP is similar to other ILDs showing up as fibrosis of lung tissue.[93]

***Treatment.*** Avoiding the causative agent is the only way to completely treat HP. However, this is not always possible as avoidance may be impossible for certain occupations or lifestyles, such as farming. If avoidance is unrealistic, treatment of symptoms may help. Corticosteroids may be used to alleviate symptoms of acute HP, but this is not a long-term solution nor does it help with chronic forms of

HP. If blood oxygen saturation falls below 90%, oxygen therapy may be used. Bronchodilators or opioids may also be used to open airways or control shortness of breath or chronic coughing, respectively, if disease has been shown to be resistant to other treatments. If the chronic form progresses to pulmonary fibrosis, lung transplantation should be considered an option but only if avoidance of the causative agent is possible. There is a much better outcome if treatment is administered in the acute stages of disease, when damage is still reversible.[93,94,109]

## FUTURE DIRECTIONS

Lung diseases with the ability to progress into more severe, irreversible forms of fibrosis pose an enormous problem to society. Not only do they effect the day-to-day life quality of those with disease, but they also create a large burden on medical and economic resources. Current diagnostic tools and treatments are far from perfect; most only treat the symptoms of disease, not the underlying inflammatory mechanisms involved in disease progression.

In the case of asthma, there has not been a new oral treatment produced in 30 years, with inhaled corticosteroid use being the most common treatment for those suffering with asthma. However, asthma remains uncontrolled in >50% of patients (adults and children) using this treatment regimen.[110] Over this same period, research was being conducted on antileukotriene and anti-IgE therapies to target specific, recognized mediators of asthma.[111] Using the targeted therapy approach, there is a need to define and decipher asthmatic phenotype. Monoclonal antibodies targeting IgE and IL-5 are currently available to treat asthma associated with TH2 lymphocytes and eosinophils. There are other biologics in clinical trials that are targeting asthma that is not linked with the TH2 response.[112] Personalized medicine utilizes 'omics' in order to determine asthmatic phenotype more specifically. Using new technology, like genomics, transcriptomics, and proteomics, can identify genes and proteins involved in asthma disease progression to not only cure asthma, but potentially prevent it.[113]

Regarding farmer's lung or hypersensitivity pneumonitis, diagnosis and treatment remain huge obstacles; there is no cure and treatments for the chronic form are minimal and ineffective. The only effective course of treatment is total avoidance of the causative agent. However, for farmers, this can cause a dramatic change in life or livelihood and is not realistic for most.[114] Systemic corticosteroids, immunosuppressive drugs, or a combination of both may be used if avoidance of the antigen is not possible, but these are not considered long-term solutions and only treat the symptoms.[115,116] If the chronic form of HP progresses to a fibrotic type, lung transplantation is the only option. However, even after transplantation, avoidance of the instigating microorganisms is needed to prevent relapse of HP.[117]

There are many questions remaining regarding all aspects of lung disease: Why do not all people exposed to the same microorganisms develop disease? What are the environmental and genetic factors involved in pathogenesis? Are there useful molecular tests available to help in diagnosis? It is obvious

that better diagnostic and treatment options are needed in regard to either of these lung diseases. Further research is needed to identify the cellular and molecular mechanisms taking place during disease progression. It is with this knowledge that patient specific treatment plans can be implemented to halt, cure, or even prevent disease.

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