I, Reshmi Indugula, hereby submit this original work as part of the requirements for the degree of Master of Science in Immunology.

It is entitled:
Fungal Exposure and Development of Autoimmune Disorders

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This work and its defense approved by:

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Committee member: Jonathan Katz, Ph.D.

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Fungal exposure and development of autoimmune disorders

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Abstract

Over the past decade there has been an alarming increase in prevalence of autoimmune diseases worldwide. They occur as a result of body’s inability to distinguish between self and non-self. Exposure to environmental microbes can play a crucial role in induction of these diseases. While controlled and targeted immune response is essential to eliminate microbial infection, uncontrolled and inappropriate immune response leads to autoimmune disorders. This review focuses on immune response to fungi and how fungal exposure can lead to autoimmune disorders. Despite disturbing health effects associated to fungal exposure, no standardized exposure limits or threshold limit value has been established yet. The nature and ubiquity of fungal materials makes it difficult to study exposure-disease relationships.
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Introduction

There are as many as 1.5 million fungal species in the environment and around 200 of them have been associated with human diseases [1,2]. Fungal infections range from relatively harmless to serious and devastating infections like invasive aspergillosis and systemic candidiasis [2]. Invasive fungal infections pose a major threat to immunocompromised individuals as well [3]. Considering the wide range of fungal pathogens human beings are constantly exposed, the infections that result from these exposures are limited [2]. Human body’s remarkable immune system protects it from these infections.

Immune system functions on its ability to distinguish self from non-self. Foreign materials like pathogens are recognized by the cells of the immune system and inflammatory responses are waged against them which often results in the clearance of pathogens. While controlled and well-organized immune responses is inevitable for eradicating harmful pathogens, uncontrolled and excessive immune responses results in self-damage and diseases. Persistent acute immune response leads to loss of self-tolerance & induction of autoimmune diseases.

1. Immune response to fungal infections

Immune response to fungal exposure varies depending upon the fungal material, immune cells and pathways that are involved.

1.1 Innate immunity

Innate immunity is the first wave of defense against infection. Recognition of fungal material by the immune cells is the initial step in activating innate immunity. There are distinct molecular structures shared by specific groups of pathogens called Pathogen Associated Molecular Patterns (PAMPs). Fungal PAMPs are recognized by a Pattern Recognition Receptors (PRRs) present on the surfaces of the immune cells. Binding of PAMPs by PRPs triggers a cascade of downstream signaling [4]. Several different PRRs may be activated on one or different cell types simultaneously leading to production of various proinflammatory molecules.

Major cells of innate immune system are neutrophils, mononuclear leukocytes (monocytes & macrophages), dendritic cells and natural killer (NK) cells. Epithelial and endothelial cells act as physical barriers against invading microbes. Neutrophils, monocytes and macrophages eradicate pathogens by phagocytosis. Neutrophils also release neutrophil extracellular traps with antimicrobial proteins, which trap and kill microbes [5]. They also kill by exposing engulfed
microbes to reactive oxygen intermediates. While neutrophils patrol the body in surveillance of microbes, monocytes usually reside in tissues.

Dendritic cells acts as a bridge between innate and adaptive immunity. They detect and bind fungal material in the peripheral tissue and present them to T cells in the lymphoid organs. They express a variety of PRRs on their surfaces and shuttle between innate and adaptive immune systems. Cytokines secreted by dendritic cells, along with other costimulatory molecules lead to priming and further activation of naive T cells into different effector T cell subsets [6].

Natural killer (NK) cell are cytotoxic lymphocytes and carry out antifungal activity through cytotoxic molecules like such as perforins, and induce apoptosis of the affected cells [7]. The infected cells secrete various chemoattractants at the site of infection, which attracts neutrophils and monocytes. These chemoattractants include cytokines, chemokines and components of complement system.

1.1.1. Fungal receptors

Toll-like receptors (TLRs) and C-type lectin receptors are the two major classes of pattern recognition receptors involved in recognition of fungal PAMPs [8]. Fungal cell wall components like chitin, mannan and β-glucans are the major molecules that are recognized by PRRs [8]. Upon binding, PRRs activate a number of signaling pathway leading to secretion of signature cytokines involved in differentiation of antifungal T cell subsets, of which Th1 and Th17 are most important, thus shaping adaptive immunity against fungi [8, 12]. Table-1 shows major cell surface PRRs, their signaling pathways and different effector T cell subsets involved in immunity against fungi.

1.1.2. Toll-like receptors.

Toll like receptors are a family of conserved cellular receptors that recognizes and bind wide range of PAMPs [9]. They are expressed in phagocytes and dendritic cells. Recognition of fungal PAMPs by TLRs lead to signaling pathways which involve adaptor proteins MyD88 and TRIF, resulting in the production of cytokines IL-10, IL-12, and type1 Interferons (IFNs) [8]. TLR-2 signaling through MyD88 activates immunomodulatory Tregs, whereas TLR-4/MyD88 signaling results in the activation of Th1 cells (8). In response to zymosan TLRs induce production of IL-6 and IL-23 which helps differentiation of Th17 cells [8]. TLRs also promote antifungal effector activities in neutrophils like respiratory burst and degranulation [9].
Table-1.Pattern recognition receptors and signaling pathways involved in fungal immunity (13, 34).

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1.1.3. **C-type lectin receptors**

Besides TLRs, C-type lectin receptors are the other major family of receptors involved in immune response against fungal pathogens. Dectin-1, Dectin-2, Macrophage-inducible C-type lectin (Mincle) and Mannose receptors are C-type lectin receptors associated with fungal pathogens.

1.1.4. **Dectin-2 and Mincle receptors**

Dectin-2 and Mincle receptors signaling involves phosphorylation of Spleen tyrosine kinase (Syk), which leads to formation of a complex of CARD9, BCL-10 and MALT1, and downstream activation of NFKB [12]. Signaling through these receptors trigger cellular responses like phagocytosis, chemotaxis, respiratory burst, cytokine production and promote differentiation of Th17 subsets [12].
1.1.5. **Dectin-1**

Dectin-1 induces multiple pathways leading to activation of Th1 and Th17 cells. In addition to above mentioned Sky/CARD9 pathway, Dectin-1 also signals through Syk-independent pathway involving Ras and Raf1 followed by downstream activation of NFkB, which results in induction of Th17 subset [8]. Dectin-1 activates NLRP3 inflammasome leading to production of inflammatory cytokine IL-1β resulting in priming of TH17 [8]. Furthermore, Dectin-1 activates Th1 subset through Syk dependent NIK pathway [8]. Fungal β-glucan are recognized by Dectin-1 receptors. In dendritic cells dectin-1-receptor mediated signaling results in production of type I INF, which confers protective immunity to the host (12).

1.1.6. **Mannose receptor**

Mannose receptor is a C-type lectin receptor expressed mainly by macrophages (11). Fungal cell wall glucans with terminal mannose are recognized by mannose receptors which lead to phagocytosis of fungal material. They promote production of proinflammatory cytokines like IL-1β, IL-6, II-12 and GM-CSF [11, 8]. Mannose receptor binding leads to downstream signaling which results in activation of IL-17 producing memory T cells [10]. IL-17 is a cytokine involved in host defense as well as induction of autoimmune diseases [11].

1.2 **Adaptive Immunity**

Events occurring during innate immunity lay foundation for development of adaptive immunity. Dendritic cells play an important role in linking innate immunity to adaptive immunity. They capture fungal antigens from the peripheral tissue and present them to the naïve T cells in lymphoid organs. This triggers the activation and commitment of naïve T cells into different effector subsets. Antigens presented on MHC class I and MHC class II molecules leads to priming of CD8+ and CD4+ T cells respectively. CD4+ cells further differentiate into different subsets called T helper (Th) cells, which include TH1, Th2, Th17 and regulatory T cells (Tregs). Th1 and Th17 subsets are predominantly involved in defense against fungi [13,14 ]. Tregs on the other hand play a regulatory role by modulating and preventing uncontrolled immune response. They produce inhibitory cytokines IL-10 and TGF-β and help maintain self-tolerance [15].

Th1 carries out antifungal defense through release of proinflammatory cytokines like INF-Y, TNF –α and GM-CSF, which results in the activation of macrophages [13]. Macrophages eradicate fungal pathogens by release of reactive oxygen intermediate and nitric oxide. Th17 subset express cytokines like IL-17A, IL-17E and IL-22, which leads to recruitment of neutrophils and release of antimicrobial peptides by keratinocytes and epithelial cells, resulting in clearance of fungal pathogens [13]. They are also the major cytokine involved in development of autoimmune diseases [39, 41].
While there are a number of studies examining innate and adaptive immunity to fungal defense, studies focusing on role played by humoral immunity is limited. Some studies show immunoglobulins confer protection either by directly targeting fungal cell wall components or by indirect mechanisms like opsonization, activation of complement pathways and antibody-directed cell toxicity [13, 16].

2. **Fungal exposure, Th17 cells and autoimmune disorders**

Autoimmune disorders occur when body's immune system designed to protect against infections inadvertently targets and destroys self-molecules. The likelihood of developing autoimmune disorders are higher in genetically susceptible individuals when exposed to specific environmental factors. Both genetic and environmental factors play important roles in development of these disorders. It has been well established that fungal exposure can trigger autoimmune diseases in mouse models.

Rheumatoid arthritis is a chronic inflammatory autoimmune disorder that affects the joints. Studies show that SKG mice which are genetically predisposed to autoimmune arthritis did not develop the disease when kept in specific pathogen free (SPF) environment [17, 18]. Upon injection of fungal β-glucans (like zymosan, curdlan and laminarin) these SKG mice kept in SPF condition readily developed chronic arthritis [17, 18].

Infection with *Candida albicans*, one of the most common fungal pathogen in humans, polarizes T cell differentiation towards TH17 subset resulting in most destructive arthritis in chronic murine SCW mouse models [4]. Under physiological conditions proinflammatory cytokines produced by Th17 help in clearing fungal pathogens, whereas in arthritic joints it causes increased inflammation, angiogenesis and osteoclastogenesis resulting in sever cartilage and bone damage [4, 19]. Numerous studies have confirmed the crucial role played by Th17 cells in development of autoimmune diseases [35, 36, 38, 39, 40].

Systemic Lupus Erythematosus (SLE) is an autoimmune disease which affects multiple organs of the body. Th17 cells play an important role in progression of SLE, both in mouse and in human models [38, 39]. Increased concentration of IL-17, signature cytokine of Th17 cells were seen in patients during SLE flare-ups [40]. Patients also exhibit elevated levels of IL-23, cytokine responsible for the development of TH17.

Multiple Sclerosis (MS) is an autoimmunun disease affecting the central nervous system due to destruction of myelin sheath protecting the nerve fibers. Autoreactive Th17 cells cause blood-brain barrier disruption and inflammation of central nervous system [41]. Affected individuals show an elevated expression of IL-17A in peripheral blood and increased presence of Th17 cells in damaged tissues [40]. Deficiency or blocking of IL17 has shown to cause delay in the onset and reduced severity of the disease.
Type-1 Diabetes is characterized by destruction of insulin producing β cell of pancreatic islets by autoreactive T cells [39]. Elevated Th17 levels were observed in peripheral blood of Type I diabetes patients as well [42]. Because and IL-17 producing TH17 cells play a crucial role in pathogenesis of various autoimmune diseases, mechanisms and pathways leading to development and proliferation of Th17 cells are potential targets for immunotherapy [39, 40, 41].

Though IL-17 producing TH17 cells are elevated in SLE, MS and Type-1 Diabetes, there is an absence of studies showing direct link between fungal exposure and elevated Th17 cells for these diseases.

3. Mechanisms that lead to development of autoimmunity

Microbial exposure can lead to autoimmunity in two major ways, either through microbial products like peptides which acts as super antigens or it can result from a prolonged and unresolved microbial infection. Fundamental mechanisms for induction of autoimmunity are molecular mimicry, epitope spreading and bystander activation [20].

3.1. Molecular mimicry

Molecular mimicry is the mechanism by which fungal antigens which are structurally similar to host antigens activate autoreactive T cell. This leads to loss of self-tolerance and immune response waged against fungal antigens cross reacts with self-antigens, resulting in tissue damage and diseases [20, 21]. Gustafson et al. shows how molecular mimicry in candida albicans mediates adhesion of the yeast to human endothelial cells. Candida albicans express a protein that is antigenically and structurally similar to adhesion molecule integrin, which helps the yeast to adhere to host tissues leading to invasive candidal infections (Fig-1),[22].

![Figure-1. Molecular mimicry. Candida albicans express a protein similar to integrin which helps it adhere to host tissue (22).](image-url)
3.2. **Epitope spreading**

Immune response by autoreactive lymphocytes do not always target one specific protein or epitope, but can be extended to include other epitopes in the same or different proteins, which is called epitope spreading [23]. An acute primary immune response causes tissue damage which exposes self-antigens which are otherwise hidden from the cells of immune system. Now these self-proteins or antigens become revealed and evoke a secondary autoimmune response which exacerbates tissue damage leading to autoimmune disease [23]. Epitope spreading is a phenomenon which follows a damaging initial inflammatory response.

3.3. **Bystander Activation**

Immune tolerance to self is not foolproof. There is a pool of autoreactive T and B cells in the body even during disease free conditions [24]. Regulatory mechanisms of the immune system keeps these autoreactive cells under control during normal conditions [25]. Bystander activation is the mechanism by which a microbial infection can activate these otherwise dormant autoreactive immune cells, initiating an autoimmune disease (Figure-2).

3.4. **Superantigens**

Some microbial antigens like cell wall components or peptides can cause large scale nonspecific activation of host immune system. They do not undergo normal antigen presenting mechanism, and activate large number of T cells, resulting in massive proliferation of T cells and production of proinflammatory cytokines [30]. Superantigens are capable of activating autoreactive B and T cells which leads to development of autoimmune diseases [31].

![Epitope spreading and bystander activation](image)

*Figure 2.* Epitope spreading and bystander activation. Dendritic cells (DC) present fungal antigens to T cells and activate them. An unresolved infection can expose self-antigens leading to activation of otherwise dormant autoreactive T cells, a process known as bystander activation.
4. *Th17/Treg balance*

Different classes of microbes evoke different lineage specific activation of naïve T cells [14]. Fungal pathogens preferentially induce T cell polarization towards Th17 lineage [14, 4]. Binding of fungal PAMPs by toll-like receptors, dectin 1- receptors and mannose receptors, all ultimately results in induction of Th17 cells. Th17 cells influence production of an array of inflammatory cytokine including IL-17A, IL-17F, IL-21 and IL-22 [26, 27].

Th17 cells has been identified as the predominant T cell lineage, underlying the development of autoimmune diseases [4, 28]. Studies have shown that expression of Th17 subset creates a positive feedback loop that favor Th17 responses, and inhibit expression of Tregs [28].

On the other hand, Tregs control or modulate immune response carried out by proinflammatory Th17 cells. They mediate self-tolerance by suppressing autoreactive effector lymphocytes and thereby inhibit autoimmunity [28]. Immunological homeostasis depends on maintaining the delicate balance between Th17 cells that promote autoimmunity and Tregs that suppresses the activity of Th17 cells [29]. Disruption of this balance results in autoimmune diseases. Dysregulation of Th17/Treg interaction has been reported in Type 1 diabetes and SLE [39, 42, 43].

5. *Fungal exposure assessment*

Accurate assessment of fungal exposure is difficult because of their diversity and ubiquity. More accurate sampling methods need to be developed to estimate fungal exposure [32]. Existing sampling methods targets different aspects of fungal concentration. For example, the two commonly used methods for quantitative assessment of fungal spores, microscopic counting and culture based enumeration technique provide two different assessment of fungal concentration [44]. While culture based methods accounts only for viable spores, microscopic counting helps estimate total spore count but fail to provide any information about their viability.

Health effects can be due to exposure to fungal spores or fragments. They do not have to be intact or viable to impart negative health effects [44]. Quantitative standards or guidelines issued by various government and private agencies for fungal exposure are mostly based on absolute or relative indoor/outdoor comparisons, and are not based on health effects [32]. There is insufficient data showing exposure-disease or dose-response relationship for fungal exposure [33].

Health relevant fungal components need to be more focused during exposure assessment. Although there are a number of studies showing health effects of β-glucan, studies focusing on components like mannan and zymosan are limited [32].
6. Conclusion

Immune response to fungi are mediated predominantly through IL-17 producing Th17 cells. Th17 cells are strongly associated with the development of a number of autoimmune diseases in humans. Inhibiting pathways and cytokines involved in the development of Th17 cell can be potential therapy for autoimmune diseases. However, the nature of fungal pathogens and lack of accurate methods to assess fungal exposure makes it difficult to study specific exposure-disease relationship.

References


