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EDITORIAL

IMPACT OF MOLD ON MAST CELL-CYTOKINE IMMUNE RESPONSE

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Molds include all species of microscopic fungi, the spores of which are small molecules, ubiquitous, mostly found in soil with higher rainfall and high humidity, in the atmosphere of urban and rural settings and in decaying vegetation. They originate from pathogenic fungi and have a crucial role in inflammatory response, causing a broad range of diseases. Immune suppressed subjects may develop mycoses caused by opportunistic common pathogenic fungi. Mast cells (MCs) are immune cells involved in the pathophysiology of infected skin, lung, and organs, where there is an increase of angiogenesis. Airways fungi infections can induce allergic lung disease mediated by MCs and other immune cells. In addition, fungal infection may cause and/or aggravate asthma inflammation. Spores are able to navigate in the airways of the lung and can be recognized through toll-like receptor (TLR) signaling by the innate immune cells including MCs. Activated MCs release preformed mediators including histamine, proteases (tryptase, chymase), pro-inflammatory cytokines/chemokines and they also generate arachidonic acid products. MCs activated by fungi provoke an increase of PGD₂ levels and lead to hypersensitivity diseases which present signs such as irritation of the respiratory tract and eyes, recurrent sinusitis, bronchitis, cough and neurological manifestations including fatigue, nausea, headaches and brain fog. Therefore, fungi activate the innate immune response through the TLRs, leading to the release of myeloid differentiation factor 88 (MyD88) which, with a series of cascade reactions, induces the stimulation of AP-1 and NF- κ B with subsequent activation of inflammatory IL-1 family members. Here, we report that fungi can activate MCs to secrete pro-inflammatory cytokines which may be inhibited by IL-37, a new anti-inflammatory IL-1 family member.

The vast majority of genes found in the human body are mainly microbes, such as bacteria, viruses and fungi. Molds are a large number of fungal species which appeared on our planet hundreds of millions

of years ago before the appearance of animals that had to develop sophisticated mechanisms of defense against these infectious agents. Molds are a group of fungi called *Hyphomycetes* that play an important

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role in the pathogenesis of plants, animals and humans, and are eukaryotic, saprophytic organisms. Mold exposure to immune compromised subjects may lead to an increased risk of lung infection, and the exposure to fungi during the first year of life may increase the risk of childhood asthma. Inhalation is considered the primary way that people are exposed to fungi, along with skin and diet. However, molds are generally not harmful to healthy humans. According to the Centers for Disease Control and Prevention, some of the most common indoor molds are *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria*, *Stachybotrys chartarum*.

Aspergillus is the most common fungus group in our environment and can cause several pathological disorders; however, in individuals with normal functioning of the immune system, infections caused by *Aspergillus* are relatively unusual (1). *Conidies*, with aerodynamic properties, are structures capable of resisting adverse environments, allowing fungi to reproduce and settle in the airways. Therefore, fungi participate in the etiology of respiratory tract allergic disease (2). Subjects living in moldy environments show an increase of 30-40% of allergy and respiratory diseases.

In the United States, *Aspergillosis* is the second cause of fungi infection and includes more than 185 species (3). For examples, the most commonly isolated and pathogenic are: *A. fumigatus*, *A. flavus* and *A. niger*; while others are less pathogenic such as *A. clavatus*, *A. glaucus*, *A. nidulans*, *A. oryzae*, *A. terreus*, *A. ustus* and *A. versicolor* (4). After *Candida*, which causes opportunistic mycosis (and MCs are the first immune cells to recognize it), *Aspergillus* species can cause different clinical situations such as opportunistic infections, allergy, and toxicity. *Aspergillus* is the most commonly isolated in invasive infections (5).

Mold-induced asthma and allergy are characterized by an augmentation of MCs and eosinophils, which generate many inflammatory mediators demonstrating to be potential effectors cells in the pathogenesis of these disorders (6).

Epithelium Toll-like receptors (TLRs) react with fungi, generating inflammatory cytokine transcription and translation (7). The host responds to fungal

invasions with self-defense mechanisms such as pattern recognition receptors (PRRs), pathogen-associated molecular patterns (PAMPs) and TLRs (8).

The Toll (which in German means extraordinary) gene was discovered by Jules Hoffmann (Nobel Prize) in the fruit fly (*Drosophila melanogaster*) and is essential for immune defenses. TLR-dependent pathways are very important for host defense against fungi and bacterial pathogens.

It is clear that phagocytes have a mechanism that can recognize mold on their surface, now known as PRRs, which have been reported to be involved in antifungal responses in immune cells. The PRR family consist of TLRs and several other molecules.

TLRs go from TLR1 to TLR13 proteins, most of which are externally expressed on the cell membrane; whereas TLR3, TLR7, TLR8, TLR9, TLR11, TLR12 and TLR13 are expressed intracellularly on endoplasmic reticulum, endosomes, phagosomes, and lysosomes. Excluding TLR3, all TLRs are involved in the activation of myeloid differentiation factor 88 (MyD88). Therefore, TLRs utilize MyD88 and the adaptor molecule IL-1 receptor-associated kinase (IRAK) complex to transduce intracellular signals.

The phosphorylation cascade engages IRAK4, TRAF6 and TAK which activate both NF- κ B and MAPK, essential for cell proliferation and inflammation.

TLR, which was first described in fruit flies by Hoffman and Beutler (2011 Nobel Prize awarded in Physiology) is utilized by APC cells to initiate a bridge between innate and adaptive immunity. TLRs recognize the pathogens and begin the immune response by activating chemotherapeutic molecules and intervening in phagocytic cells and MCs that produce inflammation. TLRs are expressed by different human cells, including MCs and macrophages, and recognize microbes, mold, macromolecules and cytokines, leading to early detection of infections. TLR is an adjuvant for the stimulation of adaptive T-cell activity and regulation of CD8⁺ T cells. It behaves differently when they need to detect pathogen-associated molecules (PAMPs) and endogenous endocrine molecules (DAMPs) (9). For example, the stimulation of

TLR2 and TLR4 leads to an increase in the release of inflammatory cytokines and plays a major role in inflammatory disorders.

Therefore, pro-inflammatory TLR signaling pathway leads to the generation of inflammatory cytokines and chemokines, including TNF and interferon regulatory factors (IRFs), via NF- κ B activation. TLR has been detected in many pathologies characterized by primary immune deficiency and infectious diseases. Mold infections, as well as bacterial infections, are associated with a defect of NF- κ B and TLR3 deficiency which can lead to infection by herpes simplex.

Intracellular TLRs (excluding TLR3) recognize the invasion agent and amplify the signal through MyD88 which is important for the generation of cytokines and the elimination of infectious agents; while transmembrane TLRs (TLR4 and TLR2) recognize LPS and peptidoglycan (10). However, the inhibition of MyD88, IRAK4 and NF- κ B may have a therapeutic effect on inflammatory diseases.

Cytokines and mold

Cytokines are a family of structurally- and functionally-related proteins that exhibit pleiotropic effects on the growth and function of a variety of cell types (11-12). Cytokines are key mediators of the host defense against mold provoking chronic inflammatory diseases (13). Cytokines play their biological effect through their trans-membrane receptors located on the cellular outer membrane triggering signal pathways.

Genetic factors, the environment and the characteristics of the fungus cause the allergic reaction, which may differ from one subject to another; this allergy is mediated by pro-inflammatory cytokines. Several mold diseases are favored by a type 1 cytokine profile associated with a type 2 cytokine. Fungi (such as *Aspergillus*, *Candidiasis*, etc.) can provoke allergy by increasing the levels of IgE and therefore the number of MCs involved in a bronchospastic reaction with an increase of IL-4, IL-5, IL-1, IL-2 (modest production) and IFN γ ; while IL-10 and IL-37 (of which we will speak below) released have a beneficial anti-inflammatory effect.

The interaction between human macrophages and MCs is of considerable interest since many macrophage

products can potentially activate MCs and *vice versa*. The recruitment of MCs into fungus-infected tissue is a crucial feature of allergic inflammation where cytokines and chemokines are generated. Therefore, fungus are involved in allergic fungal asthma which is a relevant chronic disorder mediated by MCs and other cell types and causes fibrotic remodeling of bronchi, smooth muscle increase and inflammatory cell infiltration, causing long-term morbidity (14).

Epidemiological studies in industrialized countries have shown that some molds such as *Cladosporium herbarum*, are involved in severe and persistent asthma.

The most commonly studied fungus, such as *Aspergillus*, *Alternaria*, *Botrytis*, *Cladosporium*, *Epicoccum*, *Fusarium*, and *Penicillium* species, can cause an allergic reaction and in open environments more fungal spores of Ascomycota can be found. In addition, fungal proteins are the main adjuvant factors capable of inducing a Th2 response resulting in inflammation.

It is well documented that IL-1 plays an important role in host defense versus microbes and inflammation acting through two types of receptors: IL-1R1 (stimulatory type 1), and IL-1R2 (suppressive type 2). In general, mold has an interaction with the human immune system leading to the activation of innate immune cells following the release of pro-inflammatory cytokines including IL-1 family members.

IL-1 family members react against pathogens and promote an innate immune response, however, many of them are also highly inflammatory cytokines (15). IL-1 is found in individuals with mold inflammation and immunological disorders and is thought to contribute to leukocyte migration, tissue remodeling, pain, arachidonic acid cascade (thromboxane and PGE2 synthesis in macrophages) activation and inflammation (16-17).

IL-1 is mitogenic for mesangial cells in the kidney, and glial cells in the brain, and activates keratinocytes, osteoclasts and MCs, increases fibroblast proliferation, and the synthesis of collagens and glucosaminoglycans.

IL-1 also plays an important role in the TH1 response, and requires cleavage into an active mature

molecule by the intracellular cysteine protease called caspase-1 [also known as IL-1 β -converting enzyme (ICE)]. IL-1 β shares with IL-18 significant primary amino acid sequences and induces gene expression and synthesis of TNF, Fas ligand, and several cytokines/chemokines (18). It has been reported that IL-1 activates MCs and promotes the generation of chemokines and therefore the cell recruitment influencing allergic mediator release (19).

MCs and mold

MCs have a complex immunomodulatory effect and their study has been increased in recent years. MCs develop and mature CD34⁺ pluripotent progenitor cells under the influence of IL-3, IL-6 and SCF (20).

MCs respond to many different stimuli and are usually associated to immediate hypersensitivity and allergic disorders, as well as in certain protective immune responses to microbes and fungi which bind TLRs and lead to the production of *de novo* synthesis of pro-inflammatory cytokines.

MCs also generate growth factors, leukotrienes, prostaglandins, and NO. They can be activated by IL-1, IL-33, IL-9, tryptase, and certain neuropeptides; on the other hand, cytokines such as IL-33 and IL-1 may activate MCs without degranulation (21).

MCs are present in tissues, and particularly on the skin where they play the role of "sentinels" and come into contact with fungi, and participate in both the initial and the late immune phases producing cytokines and granular proteins.

MCs generate unique cellular responses through their receptor-mediated pathways. They express receptors for the chemokine family which signal through G-coupled receptors (GPCRs) on MC membrane (22).

Moreover, MCs activated with *Candida albicans* *in vitro* release IL-8 which is a chemokine that attracts neutrophils.

Therefore, MCs can generate IL-1 and TNF which may regulate the production of eotaxin which cannot be considered specific chemoattractant (23-24).

In the light of new research on inflammation, we propose IL-37, an inhibitor of the inflammatory IL-1 family members, as a cytokine that could help mold-induced disease therapies.

IL-37 as a potential inhibitor of inflammation induced by mold

We and other Authors recently reported that IL-37 may inhibit allergy, atherosclerosis and skin inflammation. Other authors published that IL-37 reduced the inflammatory reaction in arthritis, autoimmunity, and cancer. Inhibitors of inflammatory IL-1 family member activity, such as IL-37, may be useful as Th1 immunosuppressive agents (25-26).

In recent years, according to published international consensus, using IL-37 as an inhibitor has revealed a significant reduction of inflammation mediated by macrophages and other immune cells (27-29). IL-37 (or IL-1F7) exerts an anti-inflammatory effect by binding IL-18 receptor alpha (IL-18R α) chain, and directly inhibits MyD88 (28).

IL-37 therapy is still in the embryonic stage, but we suggest that this interleukin, along with other appropriated therapies, may inhibit inflammatory Th1 cytokines in response to various stimulus including fungus and bacteria. This anti-inflammatory cytokine prevents inflammation induced by IL-1, IL-6, IL-18, IL-32 and IL-33, and it is involved in the improvement of inflammation due to *de novo* synthesis mediators generated by MC activation, reducing acute and systemic inflammatory diseases. IL-37 increases the anti-inflammatory IL-10 and owns pleiotropic effects, with significant reduction of inflammatory severity.

We propose IL-37 as a good candidate in clinical medicine to treat a broad spectrum of inflammatory diseases mediated by immune cells including macrophages and MCs, reducing the complications and mortality.

Here, we report for the first time that IL-37 can improve the physio-pathogenesis of inflammatory diseases mediated by molds, describing a new concept of inhibition and cure.

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