Effect of Mold Constituents on Innate Immune Responses (Alphabet Soup for Fungal Exposure)

Charles S. Barnes, Ph.D.
Children’s Mercy Hospitals and Clinics
The Fungal (fifth) Kingdom

• Fungi include mold, yeast, mushrooms and other growths that don’t photosynthesize. >10^6 Taxa.
• Fungi reproduce via microscopic spores or by budding (yeast).
• Fungi are usually saprophytic, preferring food that is almost or already dead.
• If conditions are right, a fungus may grow while its mammalian host is living.
• Fungi typically thrive at cooler temperatures and most do not grow well at human body temperature (notable exceptions)
• Most human fungal infections are of the skin and nails.
• Most fungal pathogens don’t produce disease in human hosts with normal immunity. Plants are fair game.
Fungal exposure is unavoidable.

• We breathe in up to 1 million spores per day during the summer.
• Soil contains millions of fungi per kilogram.
• Common foods such as bread, cheese and beer are made with fungi.
• Edible mushrooms are masses of fungal mycelia.
Fungi are very similar to human cells

- They are eukaryotes
- They contain mitochondria
- They glycosylate Proteins

Features fungal cells possess that human cells don’t.

- A thick often rigid cell wall of carbohydrate polymers and proteins
- Mannans, Chitin
- Beta-glucan molecules
Fungi are closer to mammals than bacteria.
Beta-Glucans

- Beta-Glucans are polysaccharide polymers of D-glucose monomers linked by beta-glycosidic bonds.

Ball-and-stick model of part of the crystal structure of cellulose Iβ. X-ray crystallographic data from *J. Am. Chem. Soc.* (2002) **124**, 9074–9082 This work has been released into the public domain through Wikipedia by its author Ben Mills.
Innate Immunity

• There are many aspects to innate immunity. Complement, Cytokines, Chemokines, Defensins, NK cells, macrophages, etc.

• The part of innate immunity that relates especially to fungi involves receptor molecules on the surface and interior of human immune cells that recognize and interact with the beta-glucan and other fungal specific molecules.
PRR’s, PAMPS and DAMPS

• PRR= Pattern recognition receptor. Cell surface receptors that recognize specific pathogen-associated molecular patterns.

• PAMP's= Pathogen associated molecular patterns. Molecular patterns commonly present in microbes but not in mammals. When they detect PAMPs, PRRs trigger an inflammatory response leading to destruction of the invading microorganisms.

• DAMPs endogenous =damage-associated molecular patterns
TLR’s, NLR’s, RLR’s, CLR’s and CDS’s

- TLRs Toll like receptors. Named after fruit fly Toll receptors (best characterized receptors involved in early innate immune response).
- NLR's NOD like receptors. NOD = nucleotide-binding oligomerization domain
- RLR RIG-1-Like Receptors. RIG-1 = retinoic-acid-inducible protein 1. Cytoplasmic RNA sensing receptors (antiviral).
- CLR's C-type Lectin Receptors. Fungal recognition and modulation of the innate immune response.
- CDS's Cytosolic dsDNA Sensors. Receptors to diverse molecules of microbial origin (PAMPs), or released from damaged or dying cells (DAMPs)
Major Receptors for Fungal PAMPS

- C-type lectin receptor family recognizing glucan and mannin
  - Dectin-1, Dectin-2, Mincle, SIGNR, and mannose receptor
  - Scavenger receptors such as CD5, CD36
  - Galectin-3,

- Toll-like receptor (TLR)
  - TLR2 in heterodimer combination with TLR1 or 4 or 6 recognizing fungal cell surface components.
  - TLR3, 9 and 7 recognizing fungal RNA and DNA.
  - Several TLR SNPs have significantly risk of contracting fungal infection in humans
C-type lectin receptors (CLRs) are primarily associated with fungal recognition

- CLRs are a large family of transmembrane receptors that bind to carbohydrates in a calcium-dependent manner.
- CLRs include Dectin-1, Mincle (macrophage-inducible C-type lectin), DC-SIGN (dendritic cell-specific ICAM3-grabbing nonintegrin), DMGR-1 (DC NK lectin group receptor-1) and MBL (Mannose-binding lectin).
- These CLRs share one or more CRD (carbohydrate-recognition domains).
- These receptors are involved in fungal recognition and the modulation of the innate immune response.
CLR Types

- Type I. Includes DEC 205 and MMR (macrophage mannose receptor)
- Type II. Includes Dectin-1, Dectin-2, Mincle, DC-SIGN and DNGR-1
- Soluble CLR’s include MBL
- CLR’s are expressed by most cell types including macrophages and dendritic cells.
CLR Pathways from www.invitrogen.com

From Invitrogen Web Site
Dectin-1

• Dectin-1 is a specific receptor for β-glucans [Brown GD. et al. , 2003].
• β-glucans are in the cell walls of fungi (Saccharomyces cerevisiae and Candida albicans).
• Dectin-1 has CRD connected by a stalk to the transmembrane region, followed by a cytoplasmic tail containing an ITAM-like motif. (Immunoreceptor Tyrosine-based Activation Motif)
• Dectin-1 signaling has been shown to collaborate with TLR2 signaling to enhance the responses triggered by each receptor [Gantner BN. et al., 2003].
• Furthermore, Dectin-1 can modulate cytokine expression by inducing NFAT through the Ca2+-calcineurin-NFAT pathway [Goodridge HS. et al., 2007].
Dectin-1

- Dectin-1 triggers phagocytosis and activation of Src and Syk kinases, through its ITAM-like motif.
- Syk induces the CARD9-Bcl10-Malt1 complex leading to the production of reactive oxygen species (ROS), activation of NF-κB and subsequent secretion of proinflammatory cytokines [Gross O. et al., 2006, Dennehy KM. & Brown GD., 2007].
- ROS have a direct microbicidal role in the phagosome but also can affect IL-1β secretion by activating the NLRP3 inflammasome, which in turn activates caspase-1 and permits processing of pro-IL-1β [Kankkunen P. et al., 20104].
Dectin-2

- Dectin-2 is the predominant PRR for fungal infection and the induction of Th17 immunity.
- Dectin-2 binds high mannose-type carbohydrates and was shown to be the functional receptor for α-mannans.[Drummond R. et al., 2011].
- Like Dectin-1, Dectin-2 belongs to the selective group of CLRs that link pathogen recognition to adaptive immunity.
- Similar to Dectin-1, activation of Dectin-2 triggers ROS and potassium efflux, leading to NLRP3 inflammasome activation and processing of pro-IL-1β [Sancho D & Reis E Sousa C., 2012].
Mincle, part of the Dectin-2 family

- Mincle recognizes a variety PAMPs including mycobacteria, some fungi and necrotic cells [Brown GD. 2008].
- Ligands for Mincle include fungal α-mannose, and cord factor the immunostimulatory component of Mycobacterium tuberculosis [Ishikawa E. et al., 2009].
- Mincle senses damaged cells by recognizing endogenous DAMPs. [Yamasaki S. et al., 2008].
- Mincle interacts with the Fc receptor common γ-chain (FcRγ), which triggers intracellular signaling through Syk leading to CARD9-dependent NF-κB activation.
- Syk also induces mobilization of intracellular calcium (Ca2+) and the activation of the calcineurin-NFAT pathway.
DC-SIGN and DNR-1

- DC-SIGN is involved in the recognition of several viruses (HIV-1, HCV, dengue virus, CMV, ebola virus) and other microbes of the *Leishmania* and *Candida* species.

- *M. tuberculosis*, *M. leprae*, *C. albicans*, measles virus, and HIV-1 interact with DC-SIGN to activate the Raf-1-acetylation-dependent signaling pathway and modulate TLR signaling [den Dunnen J. et al., 2008].

- DNGR-1 (CLEC9A) is particularly interesting because of its restricted pattern of expression in DCs that may be exploited for cancer therapy.

- It has recently been revealed that DNGR-1 binds damaged or dead cells via exposed actin filaments [Ahrens S et al., 2012, Zhang JG. et al., 2012]. DNGR-1 is therefore considered to be DAMPs receptor since no microbial ligand has yet been identified.
MBL

- MBL (Mannose-binding lectin) is a soluble C-type lectin.
- MBL plays a crucial role in innate immunity against yeast by enhanced complement activation and enhanced uptake of polymorphonuclear cells [Van Asbeck et al., 2008].
- MBL binds to repetitive mannose and/or N-acetylglucosamine residues on microorganisms, leading to opsonization and activation of the lectin complement pathway.
- MBL also interacts with carbohydrates on the glycoprotein (gp)120 of HIV-1. [Ji X. et al., 2005].
TLRs Toll like receptors

- In 1996, Hoffmann identified Toll as an essential part of fruit fly immunity to fungi.
- TLR4 was identified as a component LPS receptor. (Beutler and Hoffmann Nobel 2011)
- Up to 15 TLR’s have been estimated in Mammals. Not all expressed in humans.
- TLR’s generally function as heterodimers that bind PAMPS and through many steps (>25 protein intermediates, MYD88, TRAF, IRAK) activate the immune system. {Interferon regulatory factor 3 (IRF-3), nuclear factor kappa-light-chain-enhancer of activated B cells (NF kB)}
- TLR’s control inflammatory and anti-inflammatory reactions to fungal-specific PAMPs.
TLR-2 is associated with Fungi

- TLR 2 heterodimerizes with TLR 1, TOLLIP (Toll interacting protein) and others.
- It recognizes multiple glycolipids from bacteria and zymosan (Beta-glucan) from fungi.
- It acts in concert with MyD88 (Myeloid differentiation primary response gene (88))/MAL aka TIRAP (Toll interleukin receptor adapter protein).
- It is expressed on the cell surface of microglia, Schwann cells, monocytes, macrophages, dendritic cells, polymorphonuclear leukocytes (PMNs or PMLs), B cells (B1a, MZ B, B2), and T cells, including Tregs (CD4+CD25+ regulatory T cells)
Fungal PAMP-TLR interactions

- TLR2 recognizes general fungal β-glucans (Viriyakosol et al., 2005; Netea et al., 2006; Sorgie et al., 2009).
- TLR2 specifically interacts with phospholipo-mannans (PLMs) unique to C. albicans (Jouault et al., 2003).
- TLR2 recognizes unidentified ligands on A. fumigatus (Netea et al., 2003).
- TLR2/TLR1 and TLR2/TLR6 heterodimers recognize the glucuronoxylo-mannan (GXM) from Cryptococcus neoformans (Fonseca et al., 2010).
- TLR2/1 heterodimers recognize A. fumigatus both in human and mice (Rubino et al., 2012).
- TLR4 is activated upon ligation of C. albicans and C. neoformans O-linked mannans (Netea et al., 2006) (Shoham et al., 2001).
- TLR4 Ligands are also present on A. fumigatus conidia but not hyphae (Netea et al., 2003).
Bottom Line

• No genetic defects in human TLRs have been associated with a primary immune deficiency conferring increased susceptibility to fungi.
• Humans lacking MyD88, an ubiquitous signaling adaptor for TLRs, fail to show increased incidences of fungal infections.
• Certain TLR SNPs are associated with increased susceptibility to fungal disease in specific “at-risk” populations
Beyond Reactive Response (future)

- TLRs and modulation of immunity of fungi (and perhaps immunity in general)
  - TLR signaling activates emergency hematopoiesis upon microbial infections.
  - TLR signaling may participate in hematopoietic homeostasis in the absence of triggers. (Bourgeois and Kuchler, 2012)
  - Environmental microbes, including fungi may influence “shape” steady-state hematopoiesis through TLR interaction (Boiko and Borghesi, 2012)
  - Binding of TLRs and microbial PAMPs could affect both proliferation and differentiation of hematopoietic cells. (Baldridge etal., 2011; Boiko and Borghesi, 2012).
Beyond Reactive Response (future)

- TLR signaling in epithelial cells may modulate communication and cooperation between hematopoietic and non-hematopoietic immune cells. (Bourgeois and Kuchler, 2012)

- TLRs may alter the response of epithelial and innate immune cells to signals presented via non-TLR pathways, such as fungal proteases or “damage-associated molecular patterns” (DAMPs) (Moretti et al., 2008; Sorci et al., 2011)

- Host DAMPs may collaborate with PAMP-activated TLRs to modulate the outcome of the inflammatory response.

- C. albicans is uniquely recognized by TLR2 after antifungal treatment that targets and alters the cell wall (Roeder et al., 2004).
General References


• WWW.invitrogen.com

• Hardison S and Brown G. C-type lectin receptors orchestrate antifungal immunity. Nature Immunology. VOLUME 13 NUMBER 9 SEPTEMBER 2012. PP817-821