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How Fungi Have Shaped Our Understanding of Mammalian Immunology

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Research into the interaction of fungi with the host has provided significant contributions to mammalian immunology. Here, I briefly review the most notable of these contributions, starting from the time of Metchnikoff, and highlight their impact on our understanding of immunity.

Introduction

Much of our current knowledge of the mechanisms underlying the immune system have stemmed from explorations of host-microbe interactions, attributed mainly to the study of viral and bacterial pathogens. Yet fungi and their components have long been known to influence immune function, and the contributions made from the study of fungal infections are often underappreciated. In light of the substantial increase in the prevalence and severity of fungal diseases (due in large part to immunosuppressive therapy and AIDS), the study of fungal infections is receiving greater attention and yielding new insights into the immune system. While undoubtedly skewed by my own interests, for this brief perspective I have selected a few seminal contributions that have arisen from the study of fungal-host interactions and have shaped, or are shaping, our understanding of immunity.

The Earlier Contributions (1880s–2000)

Metchnikoff

Metchnikoff's study of yeast infections in Daphnia, the transparent water flea, in the 1880s is one of the earliest contributions of fungi to our understanding of modern immunology (Figure 1). Although his earlier work had already described foreign body engulfment by host cells, the large size of the fungal particles enabled Metchnikoff to account in detail the recruitment of phagocytic cells to sites where the pathogen had penetrated the flea intestinal wall and the ability of phagocytes to ingest and kill the fungal pathogen (Metchnikoff, 1884). This work not only provided strong evidence for the cellular basis of immunity, but also presented early descriptions of the ability of phagocytes to fuse (i.e., the formation of giant cells) and the ability of pathogens to overcome and kill host phagocytes.

Complement

The ability of yeast to inactivate complement was first observed in the 1900s, shortly after the existence of these serum components was recognized. This inactivation of complement by yeast particles was subsequently shown to be due to the removal of a heat-resistant component, a finding that led to the identification of properdin (now known to be a C3b-binding protein) by Pillemer and colleagues in the 1950s and the proposal that there was an alternative pathway of complement activation (Figure 1) (Pillemer et al., 1954). This discovery prompted a great deal of interest, especially as it represented one of the first examples of innate or "natural" immunity in mammals, but was highly controversial, and interest in the alternative pathway waned following Pillemer's suicide in 1957. It was not until the late 1960s that the alternative pathway of complement activation was rediscovered and became more widely accepted.

Zymosan

For their studies on complement described above, Pillemer and colleagues had developed and utilized zymosan, an insoluble yeast cell-wall-derived particle that is now the most widely used fungal particle in immunology (Pillemer and Ecker, 1941). In the late 1950s, the administration of zymosan in vivo was observed to potently stimulate the immune system, enhancing phagocytosis and clearance of foreign bodies (Benacerraf and Sebestyen, 1957). This led to an interest in zymosan as a stimulator of immune function, and the administration of these fungal particles was subsequently shown to be able to protect against conditions such as radiation stress, microbial infection, and cancer. The chemical composition of zymosan was described in 1958, subsequently leading to the identification of glucan as a major immune-stimulating component of zymosan (Di Carlo and Fiore, 1958). Since these early discoveries, zymosan has been widely used to characterize a variety of in vivo and in vitro immunological phenomena, such as inflammation, arachidonate metabolism, autoimmunity, phagocytosis, and cellular migration (Figure 1).

Toll-Like Receptors

The identification of the Toll-like receptors in the 1990s was one of the most significant immunological discoveries of the last decade, one that originated directly from the study of fungal infection. Using a mutagenesis approach, the Toll receptor was identified as a component of a key signaling pathway involved in inducing antifungal responses in *Drosophila melanogaster*. Mutations in Toll and other members of this pathway rendered flies highly susceptible to infection with *Aspergillus fumigatus*, and a scanning electron micrograph showing a fly bristling with fungal hyphae is one of the iconic images of this discovery (Figure 1) (Lemaitre et al., 1996). Similar molecules were shortly thereafter identified in mammals, and the study of these pattern recognition receptors (PRRs) has led directly to an expansion in our understanding of (and ability to exploit) the mechanisms involved in innate microbial recognition. Fungal particles also provided some insightful early steps in elucidating the function of these receptors, such as the ability of TLRs to distinguish between microbial

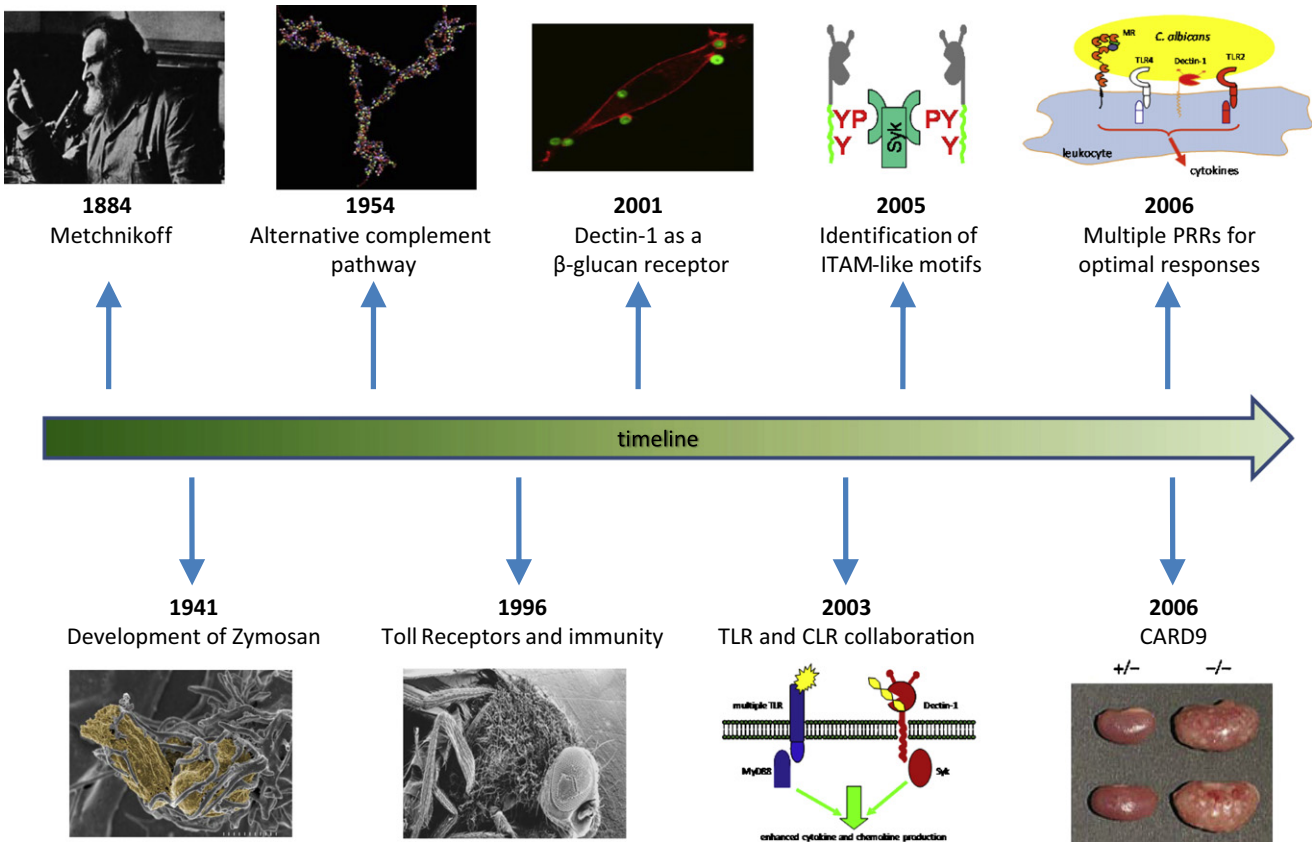

Figure 1. Contributions from Fungi to Our Understanding of Immunity

Selected seminal discoveries from 1884 to 2006 are shown along with representative images, including Metchnikoff (1884); zymosan engulfment by PMN (1941, image from Kennedy et al., 2007); properdin (1954, trimeric structure); *Aspergillus* infection in *TLR*-deficient *Drosophila* (from Lemaitre et al., 1996); fungal recognition by Dectin-1-expressing fibroblasts (from Brown and Gordon, 2001); collaborative signaling between TLR and CLRs (2003); ITAM-like motifs and Syk kinase recruitment (2005); the requirement of multiple PRRs for optimal anti-Candida responses (2006); and enhanced Candida infection in the kidneys of CARD9$^{-/-}$ mice (from Gross et al., 2006).

classes, the capacity of TLR2 to heterodimerize, and discovery of the role of TLR2 in fungal recognition.

The Recent Contributions (2000 Onward)

C-Type Lectins

The search for mammalian receptors involved in fungal recognition led to the discovery that members of the C-type lectin receptor (CLR) superfamily were also capable of triggering intracellular signaling upon microbial recognition. These receptors, of which the fungal β-glucan receptor Dectin-1 is the archetypical member (Figure 1) (Brown and Gordon, 2001), are able to induce a variety of cellular responses, such as phagocytosis, arachidonate metabolism, and respiratory burst. Importantly, like the TLRs, signaling through these receptors can induce the production of cytokines and chemokines and direct the development of adaptive immunity. Indeed, the ability of these receptors to induce Th17-type adaptive responses has been of particular recent interest. Although largely studied in the context of antifungal immunity, there is a growing appreciation that these receptors are also involved in immunity to other pathogens and in the control of homeostasis, through the recognition of endogenous ligands.

Intracellular Signaling Pathways

The study of the intracellular signaling pathways triggered by CLRs following their interactions with fungi has provided several significant insights. Perhaps most surprising was the discovery that only a single tyrosine residue in the cytoplasmic sequence of Dectin-1 was able to recruit and activate Syk kinase (Figure 1) (Rogers et al., 2005). Syk is a key kinase involved in mediating downstream signaling from many activation receptors and was previously thought to interact only with dual tyrosine-based sequences, the so-called "immunoreceptor tyrosine-based activation motifs" (ITAMs). These single tyrosine-based activation sequences or ITAM-like motifs have subsequently been identified in other receptors, which are, interestingly, all members of the C-type lectin superfamily and appear to serve largely homeostatic functions.

The identification of caspase recruitment domain 9 (CARD9) as a key downstream component of the Syk kinase pathway was another important discovery that came from studying fungal-host interactions (Figure 1) (Gross et al., 2006), and polymorphisms in this adaptor molecule and in Dectin-1 have been found to result in susceptibility to fungal infections in humans (Holland and Vinh, 2009). Mutations in this adaptor will likely be associated with more diseases, as it has since been shown to have a central role in

signaling from a variety of other non-TLR PRRs, such as NOD2 (Hsu et al., 2007) and RIG-I (Poeck et al., 2010), and has been implicated in the induction of the respiratory burst in response to bacteria (Wu et al., 2009). Other notable but perhaps less well-appreciated signaling-related discoveries arising from the study of interactions with fungi include the demonstration that Raf-1 kinase, the non-canonical NF-κB pathway, and NFAT activation are involved in PRR signaling and the description of the sequential recruitment of PRRs to phagosomes during microbial uptake (Reid et al., 2009).

Interactions between PRRs

The innate recognition of intact microbes involves multiple host receptors, and although it is apparent that the interactions between these various receptors are an important determinant of the resulting immune response, our understanding is still poor. Fungi are recognized by a large and expanding list of PRRs and are arguably one of the best models to study the interactions of different receptors. The use of these organisms has already provided valuable evidence that multiple PRRs are required for optimal antimicrobial responses (Figure 1) (Netea et al., 2006) and has given critical insights into the effects of interactions between different classes of receptor. Particularly notable were the discovery of interactions between the TLRs and CLRs upon stimulation with fungal particles and the demonstration that they could synergistically induce as well as repress the production of certain cytokines, interactions that influence the development of inflammation and adaptive immunity (Figure 1). Similar receptor interactions have subsequently been observed during the recognition of other pathogens.

Conclusions

The study of fungal-host interactions has provided seminal insights into the underlying mechanisms of immunity. As the study of fungal infections is a rapidly growing field, there are likely to be many more discoveries in the future that will further shape our understanding of mammalian immunology. Furthermore, given the high levels of mortality associated with systemic fungal diseases, despite the availability of antifungal drugs, expanding our knowledge in this area (and also how fungi evade/modulate immunity) may also provide potential avenues for adjunctive immunotherapy that could hopefully be used in treating these devastating diseases.

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REFERENCES

Benacerraf, B., and Sebestyen, M.M. (1957). Fed. Proc. *16*, 860–867.

Brown, G.D., and Gordon, S. (2001). Nature *413*, 36–37.

Di Carlo, F.J., and Fiore, J.V. (1958). Science *127*, 756–757.

Gross, O., Gewies, A., Finger, K., Schäfer, M., Sparwasser, T., Peschel, C., Förster, I., and Ruland, J. (2006). Nature *442*, 651–656.

Holland, S.M., and Vinh, D.C. (2009). N. Engl. J. Med. *361*, 1798–1801.

Hsu, Y.M., Zhang, Y., You, Y., Wang, D., Li, H., Duramad, O., Qin, X.F., Dong, C., and Lin, X. (2007). Nat. Immunol. *8*, 198–205.

Kennedy, A.D., Willment, J.A., Dorward, D.W., Williams, D.L., Brown, G.D., and DeLeo, F.R. (2007). Eur. J. Immunol. *37*, 467–478.

Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J.M., and Hoffmann, J.A. (1996). Cell *86*, 973–983.

Metchnikoff, E. (1884). Archiv f. pathologische Anatomie und Physiologie und f. klinische Medicin *96*, 177–195.

Netea, M.G., Gow, N.A., Munro, C.A., Bates, S., Collins, C., Ferwerda, G., Hobson, R.P., Bertram, G., Hughes, H.B., Jansen, T., et al. (2006). J. Clin. Invest. *116*, 1642–1650.

Pillemer, L., and Ecker, E.E. (1941). J. Biol. Chem. *137*, 139–142.

Pillemer, L., Blum, L., Lepow, I.H., Ross, O.A., Todd, E.W., and Wardlaw, A.C. (1954). Science *120*, 279–285.

Poeck, H., Bscheider, M., Gross, O., Finger, K., Roth, S., Rebsamen, M., Hannesschlager, N., Schlee, M., Rothenfusser, S., Barchet, W., et al. (2010). Nat. Immunol. *11*, 63–69.

Reid, D.M., Gow, N.A., and Brown, G.D. (2009). Curr. Opin. Immunol. *21*, 30–37.

Rogers, N.C., Slack, E.C., Edwards, A.D., Nolte, M.A., Schulz, O., Schweighoffer, E., Williams, D.L., Gordon, S., Tybulewicz, V.L., Brown, G.D., and Reis e Sousa, C. (2005). Immunity *22*, 507–517.

Wu, W., Hsu, Y.M., Bi, L., Songyang, Z., and Lin, X. (2009). Nat. Immunol. *10*, 1208–1214.