Macrophage–Microbe Interactions: Lessons from the Zebrafish Model

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Macrophages provide front line defense against infections. The study of macrophage–microbe interplay is thus crucial for understanding pathogenesis and infection control. Zebrafish (Danio rerio) larvae provide a unique platform to study macrophage–microbe interactions in vivo, from the level of the single cell to the whole organism. Studies using zebrafish allow non-invasive, real-time visualization of macrophage recruitment and phagocytosis. Furthermore, the chemical and genetic tractability of zebrafish has been central to decipher the complex role of macrophages during infection. Here, we discuss the latest developments using zebrafish models of bacterial and fungal infection. We also review novel aspects of macrophage biology revealed by zebrafish, which can potentiate development of new therapeutic strategies for humans.

Keywords: host–pathogen interactions, infection, inflammation, macrophage, zebrafish

INTRODUCTION

Macrophages are a major component of the innate immune system, responding efficiently to tissue damage and infection (1, 2). During infection, macrophages have diverse roles including phagocytosis of foreign bodies, release of cytotoxic factors, and coordination of the inflammatory response via the secretion of chemokines and cytokines (3, 4). Phagocytosis can involve the recognition of pathogen- and damage-associated molecular patterns (PAMPs and DAMPs, respectively) through pattern recognition receptors (PRRs) on the macrophage surface (5, 6). Further, the complement system can mark pathogens for phagocytosis by opsonization (7). Once internalized, the pathogen resides inside a vacuole known as a phagosome (8). Subsequent phagosome maturation involves acidification of the lumen, which leads to lysosomal fusion and degradation of the internalized microbe (9). Pathogen restriction is enhanced by the nutrient-limiting ability of the phagolysosome and the input of antimicrobial agents into the lumen, such as reactive oxygen/nitrogen species (ROS/RNS) (10). Although the majority of microbes succumb to the microbicidal environment within the phagolysosome, some pathogens (including Mycobacterium tuberculosis and Salmonella Typhimurium) can survive and replicate within this harsh environment (11, 12). In contrast, some bacterial pathogens (including Listeria monocytogenes and Shigella flexneri) have mechanisms to escape from the phagosome and proliferate in the cytosol (13).

Mechanisms of cell-autonomous immunity are crucial for protection of the host cell cytosol (14). Autophagy is an evolutionarily conserved process of intracellular degradation, recognized as an important defense mechanism against intracellular pathogens (15). Targeting of bacterial pathogens by the autophagy machinery is often mediated by ubiquitination, a posttranslational modification (16, 17). In this case, ubiquitinated substrates (such as bacterial components or damaged membrane) are recognized by autophagy receptors, including p62 and NDP52, which direct formation of the autophagic membrane around the targeted pathogen (18–20). Autophagy-related (ATG) proteins also direct immunity-related GTPases (IRGs) and guanylate-binding
proteins (GBPs) to pathogens (21). IRGs and GBPs belong to a family of GTPases that confer host cell resistance during infection by pathogens (22–24). IRGs cooperate with GBPs to target non-self vacuoles, trafficking nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, ATG proteins, and inflammasome complex assembly for host defense (25–27). Intracellular pathogens are also detected via nucleotide-binding oligomerization domain-like receptors (NLRs), a class of PRRs that reside in the cytosol (28). An important example is NLRP3, which acts as a scaffold protein for inflammasome assembly, leading to caspase-1 activation and maturation of pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18 (29, 30). These cytokines enhance the immune response and induce pathways leading to pyroptosis, a highly inflammatory type of programmed cell death (31).

A variety of different animal models have made important contributions to the study of macrophage-microbe interplay in vivo. Originally used for studying development, the zebrafish has many similarities with higher vertebrates (including mammals), which has led to their use for studying infection and immunity (32, 33). During the first 4 weeks of development, zebrafish lack adaptive immunity and rely on the innate immune response for host defense (34). This, together with ex utero development of embryos, necessitates the rapid development of innate immune cells, progenitors of which can be observed as early as 20 h postfertilization (hpf). Although the precise sites of development and maturation differ between zebrafish and human phagocytes, zebrafish macrophages retain close morphological and functional similarities with their mammalian counterparts (35). Primitive macrophages, originally identified by Philippe Herbomel and colleagues, use phagocytosis to control infection by Escherichia coli and Bacillus subtilis (36). Optical accessibility during early life stages make zebrafish larvae highly suited for non-invasive live microscopy. Studies on the zebrafish immune system identified macrophage-specific genes (including mpeg1 and csf1ra), discoveries that enabled the development of specific reporter lines (37, 38). By using transgenic lines that fluorescently label distinct leukocyte populations, studies have identified key roles for macrophages during infection control in vivo (6). Furthermore, the sequenced zebrafish genome and the ability to manipulate the immune system through chemical or genetic means (including morpholino oligonucleotides for transient depletion, or CRISPR/Cas9 for genome engineering) make the zebrafish a unique and powerful tool for studying host–pathogen interactions at the molecular, cellular, and whole-animal level (39–41).

In this review, we discuss novel aspects of host–pathogen interactions that have been recently revealed using bacterial (mycobacteria, Gram-positive, Gram-negative) and fungal zebrafish infection models, highlighting key roles for macrophages in host defense against a variety of important pathogens.

**ZEBRAFISH MACROPHAGE–BACTERIA INTERACTIONS IN VIVO**

*Mycobacterium marinum* is a natural pathogen of zebrafish, closely related to the causative agent of human tuberculosis (*M. tuberculosis*). Pioneering studies have shown that *M. marinum* infection leads to the aggregation of macrophages into granuloma-like structures that both contain and promote bacterial dissemination (Figure 1A) (42, 43). These structures are initiated by the ESX-1 secretion system, which induces expression of matrix metalloproteinase-9 in epithelial cells to recruit macrophages for bacterial phagocytosis (44, 45).

Tissue-resident macrophages first responding to infection are microbicidal. Therefore, to create a replication niche, *M. marinum* induces chemokine (C-C motif) ligand 2 (CCL2) expression and recruits uninfected monocytes via the surface lipid phenolic glycolipid (PGL) (46). Moreover, *M. marinum* possess cell surface-associated phthiocerol dimycoceroserate lipids, which impede PAMP–TLR interactions and prevent the microbicidal response in newly recruited monocytes (47). Consistent with a protective niche, the granuloma supports bacterial growth, alteration of granuloma structure through disruption of E-cadherin increases immune cell accessibility, and reduces bacterial burden (48). Work has shown that macrophage deficiency leads to accelerated necrosis of the granuloma and increased susceptibility to infection (45). A balanced inflammatory response is crucial to prevent necrosis of the granuloma, as both low and high levels of tumor necrosis factor (TNF) can lead to increased bacterial replication (49). Supporting this, a forward genetic screen performed in zebrafish revealed that mutation of the ita4h locus (encoding leukotriene A4 hydrolase) can modulate production of anti- and pro-inflammatory lipid mediators and susceptibility to mycobacterial infection (50). Angiogenesis has also been implicated in granuloma expansion and bacterial dissemination via a mechanism that requires hypoxia-induced vascular endothelial growth factor expression and C-X-C chemokine receptor type 4 (CXCR4) signaling (51, 52). Collectively, these studies highlight a complex role for macrophages in granuloma formation and in host defense against mycobacteria.

Infection by *Mycobacterium leprae*, an ancient pathogen that causes leprosy, is restricted to humans and nine-banded armadillos (*Dasypus novemcinctus*) (57). *M. leprae* infection causes demyelination of peripheral nerves and axonal damage, which can lead to symptoms such as muscle weakness and numbness. Remarkably, new work has shown that zebrafish can be used to study *M. leprae* pathogenesis in vivo (58). Although *M. leprae* replication is not observed in zebrafish due to its long doubling time of 12–15 days, the macrophage response to bacteria is comparable to that observed during *M. marinum* infection. In the case of *M. leprae*, PGL-1 is responsible for mediating structural changes in myelin by inducing macrophage RNS production, which subsequently causes mitochondrial swelling, demyelination, and axonal damage.

*Listeria monocytogenes*, a Gram-positive foodborne pathogen, can cause listeriosis and meningitis in immunocompromised individuals, and spontaneous abortions during pregnancy (59, 60). Inside macrophages, *L. monocytogenes* can escape from the phagosome and proliferate in the cytosol (Figure 1B) (61). Bacterial escape from the phagosome to the cytosol is linked to expression of listeriolysin O (LLO), a pore-forming toxin that targets the phagosomal membrane (62). ActA, another major
virulence factor of *Listeria*, enables actin tail polymerization and autophagy escape (59, 63, 64). In agreement with studies performed *in vitro* using tissue culture cells, virulence of *Listeria* in zebrafish is dependent on LLO and ActA (53). More recent work using zebrafish has shown that bacterial dissemination (*via* necrosis of infected macrophages and release of bacterial-containing blebs) is LLO-dependent (65). To counteract this, Gp96 (an endoplasmic reticulum chaperone) can protect the integrity of the host cell plasma membrane against pore-forming toxins. In a separate study, zebrafish infection with a *Listeria* strain ectopically expressing flagellin (called *Lm*-pyro) was shown to activate the inflammasome in macrophage and reduce infection (66). These results highlight the inflammasome as crucial for protection against *Listeria*.
Staphylococcus aureus is an opportunistic pathogen, which latently resides in one-third of humans. Invasive surgery or lesions can increase the risk of infection, and considering the emergence of antibiotic-resistant strains, S. aureus is recognized as a major human threat. While systemic S. aureus infections are controlled, zebrafish are susceptible to yolk sac infection (i.e., a site inaccessible to leukocytes), underscoring the importance of leukocytes for infection control (67). Experiments performed using larvae depleted of myeloid cells demonstrate a role for macrophages in restriction of S. aureus proliferation in the blood (67). Interestingly, the incomplete clearance of bacteria by leukocytes can result in an “immunological bottleneck,” viewed to select for persisting bacterial populations (68). The zebrafish can, therefore, be used to discover mechanisms used by S. aureus to evade destruction within leukocytes.

Colonization of humans by Burkholderia cenocepa ha has severe consequences in cystic fibrosis (CF) patients and other immunocompromised individuals. Originally viewed to form a biofilm in CF patients, studies using clinical samples have shown that B. cenocepa can reside in alveolar macrophages (69). More recently, a zebrafish infection model demonstrated that macrophages are crucial for B. cenocepa replication (70). Consistent with this, depletion of macrophages from larvae restricts bacterial replication (71). During infection, macrophages express IL-1β and recruit uninfected cells to form cellular aggregates (70, 71). Paradoxically, the depletion of IL-1β (using morpholino oligonucleotide) during B. cenocepa infection results in decreased survival, yet, inhibition of IL-1β signaling (using the IL-1 receptor antagonist anakinra) results in increased survival (71). Together, these experiments suggest the precise role of IL-1β during B. cenocepa infection, and its manipulation for therapy, is complex.

Salmonella is a well-studied Gram-negative pathogen responsible for gastroenteritis, enteric fever, and bacteremia. Investigation of S. Typhimurium has made important contributions to macrophage biology (6, 11). During zebrafish infection, S. Typhimurium can replicate within macrophages and also extracellularly within the vasculature (72). A subpopulation of intracellular bacteria is lysed by mitochondrial-derived ROS produced by macrophages via a pathway dependent on immunoreponsive gene 1 (IRG1) (73). In addition, macrophages are responsible for the “fine-tuning” of the immune response to S. Typhimurium via secretion of granulocyte-colony stimulating factor (G-CSF), which in turn stimulates the transcription factor C/ebpβ and enhances neutrophil production by emergency granulopoiesis (74).

Shigella is a Gram-negative enteroinvasive pathogen classified by the WHO as a global threat due to its development of antibiotic resistance (75–77). Among the species of Shigella, S. flexneri is best recognized as a paradigm for studying macrophage cell death (78). In agreement with studies performed in vitro, S. flexneri can induce cell death in zebrafish macrophages in vivo (Figure 1C) (54). Despite this, macrophage-depleted transfgenic zebrafish present increased mortality during infection (79). These results suggest that macrophages play an important role in the initial collection of injected bacteria, prior to the elimination of bacteria and cellular debris by neutrophils. The increasing risk of multidrug-resistant bacteria has driven the need for treatments that do not strictly rely on antibiotics. Injection of Shigella-infected zebrafish with predatory bacteria Bdellovibrio bacteriovorus revealed a synergy between predator–prey interactions with the host immune system to restrict multidrug-resistant infection (80). In this case, the reduction of Shigella burden by Bdellovibrio is beneficial for infection control by zebrafish leukocytes.

**ZEBAFISH MACROPHAGE–FUNUS INTERACTIONS IN VIVO**

Invasive fungal infections are a growing problem, causing significant morbidity and mortality in organ transplant patients. Immunosuppression using calcineurin inhibitors is a common strategy for the prevention of organ transplant rejection and increases the risk of infection by Aspergillus fumigatus (81). Alveolar macrophages and inflammatory monocytes in the murine lung have been described as critical for early antifungal immunity during Aspergillus infection (82). Real-time visualization using a zebrafish infection model revealed the inability of neutrophils to phagocytose fungal spores, and suggested macrophages as crucial for host defense against A. fumigatus (83, 84). In mouse models of A. fumigatus infection, treatment with calcineurin inhibitor FK506 leads to increased mortality (85). Consistent with this, studies using zebrafish infection showed a role for calcineurin in protection against Aspergillus (86). In this case, calcineurin activation leads to dephosphorylation of nuclear factor of activated T cells (NFAT), and FK506 treatment impairs neutrophil recruitment because of reduced TNF-α production by macrophages (86). A separate study revealed FK506 inhibits the calcineurin-dependent lateral transfer of A. fumigatus from necroptotic to naïve macrophages, allowing fungal escape and unrestricted growth (87). Collectively, these studies highlight the indispensable role of calcineurin in macrophages for Aspergillus control in vivo.

Candida albicans is an opportunistic fungal pathogen, which primarily affects immunocompromised individuals. Zebrafish infection models have been used to identify C. albicans virulence factors and indicate an important role for the filamentous (hyphal) form of C. albicans in pathogenesis (88–90). Strikingly, real-time microscopy of C. albicans infection showed the extrusion of hyphae from the zebrafish hindbrain (88). Fungal dissemination is observed by 24 h postinfection (hpi), followed by lethality resulting from uncontrolled hyphal growth (89). Here, macrophages can restrict germination (but not replication), and fungal killing by macrophages and neutrophils is a rare occurrence (89). Zebrafish infection has also demonstrated a new role for NADPH oxidase in controlling hyphal growth, helping to recruit macrophages through ROS and preventing germination (89, 91).

Another opportunistic fungal pathogen, Cryptococcus neoformans can be fatal in immunocompromised individuals and is responsible for over 600,000 deaths globally per annum (92). Although highly informative, mammalian and non-vertebrate infection models have limitations in visualizing fungus-leukocyte dynamics and the translatability to higher vertebrate models,
respectively. Live imaging of zebrafish during *Cryptococcus* infection revealed macrophages are required for pathogen control (Figure 1D) (55, 93). In agreement with this, macrophage depletion prior to infection leads to uncontrolled fungal replication and increased zebrafish mortality (55, 93). Macrophage-depletion postinfection also leads to increased fungal burden (55). The *Cryptococcus* capsule is made of polysaccharides contributing immunosuppressive functions, including the inhibition of phagocytosis. Consistent with this, capsule enlargement that occurs during infection of zebrafish can prevent phagocytosis, resulting in fungal proliferation and zebrafish mortality (55). By tracking individual macrophages over time, the first in vivo observation of vomocytosis (the controlled non-lytic expulsion of pathogens from phagocytes) was captured (55). The precise role of vomocytosis in host defense is not yet known.

*Mucor circinelloides* is an emerging fungal pathogen in which the incidence of infection is increasingly associated with aging populations (94). *M. circinelloides* causes mucormycosis, a disease with a wide range of symptoms including fever and gastrointestinal bleeding (95). Treatment of mucormycosis in humans remains costly and unsuccessful, and fatalities are often linked to corticosteroid treatment and immune defects (96, 97). In agreement with this, immunosuppression by corticosteroid treatment results in increased zebrafish mortality (56). Moreover, macrophage-depleted zebrafish succumb to infection, highlighting a key role for macrophages in *M. circinelloides* control. Remarkably, macrophages accumulate around viable spores in a manner similar to the granuloma structures described for *M. marinum* infection (Figure 1E) (56). While the role of macrophage clusters during *M. circinelloides* infection is not fully known, the zebrafish infection model can provide a novel platform to study macrophage–fungal interplay during mucormycosis.

**DISCUSSION**

Here, we describe recent mechanistic insights into the macrophage response to intracellular pathogens as revealed by zebrafish infection (Table 1). Although macrophage recruitment and phagocytosis is typically observed in response to infection, this is not always followed by pathogen restriction. Zebrafish infection has shown that, in some cases, macrophages can promote pathogenesis by shielding the pathogen from immune control or by providing a replicative niche. The zebrafish is a relatively new model for the study of human infectious disease. Therefore, a limitation of the system includes the lack of tools currently available, such as zebrafish antibodies and cell lines, which can impede in-depth mechanistic studies. On the other hand, the rapid development of transgenic zebrafish lines with fluorescently tagged proteins/cells, in combination with genome-editing technologies, compensate for these limitations. Considering advancements in RNAseq and high-resolution microscopy, we can expect that zebrafish infection will continue to illuminate fundamental aspects of host–pathogen interactions at the molecular, cellular, and whole animal level. The hope is that studying macrophage–microbe interactions *in vivo* using the zebrafish model can deliver therapeutic impact in humans.
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REFERENCES
1. Petrovskis G, Zabuczyk GB, Mája GN, Fésüs LS. Phagocytosis of cells dying through autophagy evokes a pro-inflammatory response in macrophages. Autophagy (2014) 3(5):508–10. doi:10.4161/auto.4731
2. Kapellos TS, Taylor L, Lee H, Cowley SA, James WS, Iqbal AJ, et al. A novel real time imaging platform to quantify macrophage phagocytosis. Biochem Pharmacol (2016) 116:107–19. doi:10.1016/j.bcp.2016.07.011
3. Pirskanen A, Gosselin-Clermont V, Burdick K, Lu X, Zhang J, et al. Microtubule-driven autophagy promotes a pro-inflammatory response in macrophages. J Immunol (2017) 197(3):1180–91. doi:10.4049/jimmunol.1600615
4. Aderem A. Phagocytosis and the inflammatory response. J Infect Dis (2003) 187(Suppl2):S340–5. doi:10.1086/374747
5. Torraca V, Masud S, Spaink HP, Meijer AH. Macrophage-pathogen interactions in infectious diseases: new therapeutic insights from the zebrafish host model. Dis Model Mech (2014) 7(7):785–97. doi:10.1242/dmm.015594
6. Flannagan RS, Maumolleau V, Grinstein S. The cell biology of phagocytosis. Ann Rev Pathol (2012) 7(1):61–98. doi:10.1146/annurev-pathol-011811-132445
7. Yeh BM, Kwon EJ, Kim MI, Shin BS, Park YW, et al. Autophagy as a metabolic reprogramming mechanism during macrophage phagocytosis. J Biol Chem (2017) 292(9):3728–41. doi:10.1074/jbc.m116.772826
8. Novoa B, Figueras A, del Barrio L, Re F. Role of the inflammasome, IL-1β, and IL-18 in bacterial infections. ScientificWorldJournal (2010) 20(1):34–50. doi:10.1038/cr.2009.139
9. Geddes K, Magalhães JG, Andrianopoulos A, Lieschke GJ. Innate immunity: beyond the innate immune response. Nat Rev Drug Discov (2009) 8(6):645–75. doi:10.1038/nrd2783
10. Uemura T, Yamagishi M, Kamata N, Hoshino K, Kawai T, et al. Autophagy as a host defense mechanism against intracellular bacterial pathogens. J Exp Med (2010) 207(11):2341–51. doi:10.1084/jem.20101505
11. Li P, Jiang W, Yu Q, Liu W, Zhou P, Li J, et al. Ubiquitination and degradation of GBP1 by a Shigella effector to suppress host defence. Nature (2017) 551(7680):378–83. doi:10.1038/nature24467
12. Meunier E, Broz P. Interferon-inducible GTPases in cell autonomous and innate immunity. Cell Microbiol (2015) 18(2):168–80. doi:10.1111/cmi.12546
13. Ray K, Marteyn B, Sansonetti PJ, Tang CM. Life on the inside: the intracellular lifestyle of cytosolic bacteria. Nat Rev Microbiol (2009) 7(10):781–95. doi:10.1038/nrmicro2346
14. Sato Y, Kepler T, Kaneko M, Goto S, Miyake K, et al. Autophagy is required for Shigella flexneri to evade host clearance. EMBO J (2012) 31(3):430–42. doi:10.1038/emboj.2011.226
15. Arango Duque G, Descoteaux A. Macrophage cytokines: involvement in host-parasite interactions in infectious diseases: new therapeutic insights from the zebrafish host model. Dis Model Mech (2014) 7(7):785–97. doi:10.1242/dmm.015594
16. Funato K, Endo K, Takamori T, Fujita T, Iwahashi M, et al. Expression of NOD-like receptors. Cell Mol Life Sci (2010) 67(2):249–62. doi:10.1007/s00018-009-0024-8
17. Anderton C, Underhill DM. Mechanisms of phagocytosis in macrophages. Ann Rev Immunol (1999) 17(1):593–623. doi:10.1146/annurev.immunol.17.1.593
18. Flannagan RS, Maumolleau V, Grinstein S. The cell biology of phagocytosis. Ann Rev Pathol (2012) 7(1):61–98. doi:10.1146/annurev-pathol-011811-132445
19. Yin X, Yang H, Wang X, Li Y, Wu X, et al. Autophagy inhibits motility of Shigella flexneri. PLoS Pathog (2013) 9(6):e1003414. doi:10.1371/journal.ppat.1003414
20. Franchi L, Warner N, Viani K, Nunez G. Function of NOD-like receptors in microbial recognition and host defense. Immunol Rev (2009) 227(1):106–28. doi:10.1111/j.1600-065X.2008.00734.x
21. Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. Nature (2011) 469(7330):325–35. doi:10.1038/nature09782
22. Kwon EJ, Kwon E, Kim MI, Shin BS, Park YW, et al. Autophagy as a metabolic reprogramming mechanism during macrophage phagocytosis. J Biol Chem (2017) 292(9):3728–41. doi:10.1074/jbc.m116.772826
23. Gomes LC, Dikic I. Autophagy in antimicrobial immunity. Mol Cell (2014) 54(2):224–33. doi:10.1016/j.molcel.2014.03.009
24. Clough B,rickel E-M. The Toxoplasma parastrophophorous vacuole: an evolving host-parasite frontier. Trends Parasitol (2017) 33(6):473–88. doi:10.1016/j. pt.2017.02.007
25. Kao H, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, et al. The zebrafish reference genome sequence and its relationship to the human genome. Nature (2013) 496(7446):498–503. doi:10.1038/nature12111
40. Blum M, De Robertis EM, Wallingford JB, Niehrs C. Morpholinos: anti-sense and sensibility. Dev Cell (2015) 35(2):145–9. doi:10.1016/j.devcel.2015.09.017

41. Li M, Zhao L, Page-McCaw PS, Chen W. Zebrafish genome engineering using the CRISPR-Cas9 system. Trends Genet (2016) 32(12):815–27. doi:10.1016/j.tig.2016.10.005

42. Davis JM, Clay H, Lewis JL, Gholi N, Herbomel P, Ramakrishnan L. Real-time visualization of mycobacterium-macrophage interactions leading to initiation of granuloma formation in zebrafish embryos. Immunity (2002) 17(6):693–702. doi:10.1016/S0959-4947(02)00475-2

43. Davis JM, Ramakrishnan L. The role of the granuloma in expansion and dissemination of early tuberculous infection. Cell (2009) 136(1):37–49. doi:10.1016/j.cell.2008.11.014

44. Volkman HE, Pozos TC, Zheng J, Davis JM, Rawls JF, Ramakrishnan L. Tuberculous granuloma induction via interaction of a bacterial secreted protein with host epithelium. Science (2010) 327(5964):466–9. doi:10.1126/science.1179663

45. Pagán AJ, Yang CT, Cameron J, Swaim LE, Ellett F, Lieschke GJ, et al. Myeloid phenotype factors promote resistance to mycobacterial infection by curtailting granuloma necrosis through macrophage replenishment. Cell Host Microbe (2015) 18(1):15–26. doi:10.1016/j.chom.2015.06.008

46. Cambier CJ, O'Leary SM, O'Sullivan MP, Keane J, Ramakrishnan L. Phenolic glycolipid facilitates mycobacterial escape from microbicial tissue resident macrophages. Immunity (2017) 47(3):552–65.e4. doi:10.1016/j.immuni.2017.08.003

47. Cambier CJ, Takaki KK, Larson RP, Hernandez RE, Tobin DM, Urdahl KB, et al. Mycobacteria manipulate macrophage recruitment through coordinated use of membrane lipids. Nature (2013) 505(7482):218–22. doi:10.1038/science.1179663

48. Cronan MR, Beerman RW, Rosenberg AF, Johnson MG, Oehlerls SH, et al. Macrophage epithelial reprogramming underlies mycobacterial granuloma formation and promotes infection. Immunity (2016) 45(4):861–76. doi:10.1016/j.immuni.2016.09.014

49. Roca PJ, Ramakrishnan L. TNF dually mediates resistance and susceptibility to mycobacteria via mitochondrial reactive oxygen species. Cell (2013) 153(3):321–34. doi:10.1016/j.cell.2013.03.022

50. Tobin DM, Vary JC, Ray JP, Walsh GS, Dunstan SJ, Bang ND, et al. The Ita4h locus modulates susceptibility to mycobacterial infection in zebrafish and humans. Cell (2010) 140(5):717–30. doi:10.1016/j.cell.2010.02.013

51. Oehlerls SH, Cronan MR, Scott NR, Thomas MI, Okuda KS, Walton EM, et al. Interception of host angiogenic signalling limits mycobacterial growth. Nature (2015) 517(7536):612–5. doi:10.1038/nature13967

52. Torraca V, Tulotta C, Snaraj-Jagalska BE, Mejier AH. The chemokine receptor CXCR4 promotes granuloma formation by a mycobacteria-induced angiogenesis programme. Sci Rep (2017) 7:45961. doi:10.1038/srep45961

53. Levraud J-P, Disson O, Kissa K, Bonne I, Cossart P, Herbomel P, et al. Real-time observation of Listeria monocytogenes-phagocyte interactions in living zebrafish larvae. Infect Immun (2009) 77(9):3651–60. doi:10.1128/IAI.00408-09

54. Mostowy S, Boucontet L, Mazon Moya MJ, Sirtianni A, Boudinot P, Hollinshead M, et al. The zebrafish as a new model for the in vivo study of Shigella flexneri interaction with phagocytes and bacterial autopox. PLoS Pathog (2013) 9(9):e1003588. doi:10.1371/journal.ppat.1003588

55. Bojarczuk A, Miller KA, Hotham R, Lewis A, Ogryzko NV, Kamuyuango AA, et al. Cryptococcus neoformans intracellular proliferation and capsule size determines early macrophage control of infection. Sci Rep (2016) 6:21489. doi:10.1038/srep21489

56. Voelz K, Gratapac RL, Wheeler RT. A zebrafish larval model reveals early tissue-specific innate immune responses to Mucor circinelloides. Dis Model Mech (2015) 8(11):1375–88. doi:10.1242/dmm.019992

57. Balamayooran G, Pena M, Sharma R, Truman RW. The armadillo as an animal model and reservoir host for Mycobacterium leprae. Clin Dermatol (2015) 33(1):108–15. doi:10.1016/j.clindermatol.2014.07.001

58. Madigan CA, Cambier CJ, Kelly-Scumpia KM, Scumpia PO, Cheng TY, Zailia J, et al. A macrophage response to Mycobacterium leprae phenolic glycolipid induces nerve damage in leprosy. Cell (2017) 170(5):973–85.e10. doi:10.1016/j.cell.2017.07.030

59. Cossart P. Illuminating the landscape of host-pathogen interactions with the bacterium Listeria monocytogenes. Proc Natl Acad Sci U S A (2011) 108(49):19484–91. doi:10.1073/pnas.1112371108

60. David DJV, Cossart P. Recent advances in understanding Listeria monocytogenes infection: the importance of subcellular and physiological context. PLoS Pathog (2017) 13(6):e1006467. doi:10.1371/journal.ppat.1006467
80. Willis AR, Moore C, Mazon-Moya M, Krokowski S, Lambert C, Till R, et al. Injections of predatory bacteria work alongside host immune cells to treat Shigella infection in zebrafish larvae. *Curr Biol* (2016) 26(24):3343–51. doi:10.1016/j.cub.2016.09.067

81. Sugui JA, Kwon-Chung KJ, Juvvadi PR, Latgé J-P, Steinbach WJ. *Aspergillus fumigatus* and related species. *Cold Spring Harb Perspect Med* (2014) 5(2):a019786. doi:10.1101/cshperspect.a019786

82. Espinosa V, Jhingran A, Dutta O, Kasahara S, Donnelly R, Du P, et al. Inflammatory monocytes orchestrate innate antifungal immunity in the lung. *PLoS Pathog* (2014) 10(2):e1003940. doi:10.1371/journal.ppat.1003940

83. Knox BP, Deng Q, Rood M, Eickhoff JC, Keller NP, Huttenlocher A. Distinct innate immune phagocyte responses to *Aspergillus fumigatus* conidia and hyphae in zebrafish larvae. *Eukaryot Cell* (2014) 13(10):1266–77. doi:10.1128/EC.00080-14

84. Knox BP, Huttenlocher A, Keller NP. Real-time visualization of immune cell clearance of *Aspergillus fumigatus* spores and hyphae. *Fungal Genet Biol* (2017) 105:52–4. doi:10.1016/j.fgb.2017.05.005

85. Herbst S, Shah A, Carby M, Chusney G, Kikkeri N, Dorling A, et al. A new and clinically relevant murine model of solid-organ transplant aspergillosis. *Dis Model & Mech* (2013) 6(3):643–51. doi:10.1242/dmm.010330

86. Herbst S, Shah A, Mazon-Moya M, Marzola V, Jensen B, Reed A, et al. Phagocytosis-dependent activation of a TLR9-BTK-calcineurin-NFAT pathway co-ordinates innate immunity to *Aspergillus fumigatus*. *EMBO Mol Med* (2015) 7(3):240–58. doi:10.15252/emmm.201404556

87. Shah A, Kannambath S, Herbst S, Rogers A, Sorens S, Carby M, et al. Calcineurin orchestrates lateral transfer of *Aspergillus fumigatus* during macrophage cell death. *Am J Respir Crit Care Med* (2016) 194(9):1127–39. doi:10.1164/rccm.201601-0070OC

88. Chao CC, Hsu PC, Jen CF, Chen IH, Wang CH, Chan HC, et al. Zebrafish as a model host for *Candida albicans* infection. *Infect Immun* (2010) 78(6):2512–21. doi:10.1128/IAI.01293-09

89. Brothers KM, Newman ZR, Wheeler RT. Live imaging of disseminated candidiasis in zebrafish reveals role of phagocyte oxidase in limiting filamentous growth. *Eukaryot Cell* (2011) 10(7):932–44. doi:10.1128/EUK.05005-11

90. Chen YZ, Yang YL, Chu WL, You MS, Lo HJ. Zebrafish egg infection model for studying *Candida albicans* adhesion factors. *PLoS One* (2015) 10(11):e0143048. doi:10.1371/journal.pone.0143048

91. Brothers KM, Gratacap RL, Barker SE, Newman ZR, Norum A, Wheeler RT. NADPH oxidase-driven phagocyte recruitment controls *Candida albicans* filamentous growth and prevents mortality. *PLoS Pathog* (2013) 9(10):e1003634. doi:10.1371/journal.ppat.1003634

92. Chen YZ, Yang YL, Chu WL, You MS, Lo HJ. Zebrafish egg infection model for studying *Candida albicans* adhesion factors. *PLoS One* (2015) 10(11):e0143048. doi:10.1371/journal.pone.0143048

93. Tenor JL, Oehlers SH, Yang JL, Tobin DM, Perfect JR. Live imaging of host-parasite interactions in a zebrafish infection model reveals cryptococcal determinants of virulence and central nervous system invasion. *MBio* (2015) 6(3):e01425–15. doi:10.1128/mBio.01425-15

94. Ibrahim AS, Kontoyiannis DP. Update on mucormycosis pathogenesis. *Curr Opin Infect Dis* (2013) 26(6):508–15. doi:10.1097/QCO.0000000000000008

95. Pak J, Tucci VT, Vincent AL, Sandin RL, Greene JN. Mucormycosis in immunocompromised patients. *J Emerg Trauma Shock* (2008) 1(2):106–13. doi:10.4103/0974-2700.42203

96. Waldorf AR, Levitz SM, Diamond RD. In vivo bronchoalveolar macrophage defense against *Rhizopus oryzae* and *Aspergillus fumigatus*. *EMBO Mol Med* (2015) 7(3):240–58. doi:10.15252/emmm.201404556

97. Ibrahim AS, Edwards JE Jr, Bryant R, Spellberg B. Economic burden of mucormycosis in the United States: can a vaccine be cost-effective? *Med Mycol* (2009) 47(6):592–600. doi:10.1080/13693780802326001

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