Pearls

Microbial Egress: A Hitchhiker's Guide to Freedom

Ana Traven*, Thomas Naderer*

Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia

The Restaurant at the End of the Infection—Macrophages as Host Cells

After colonization or invasion, intracellular pathogens seek host cells to establish infections and facilitate replication. This lifestyle provides essential nutrients and protection from immune systems. Several host cells—in particular professional phagocytes, such as monocytes, macrophages, and neutrophils—have developed antimicrobial mechanisms to deplete the replicative niche of these intracellular microbes. Programmed cell death pathways are frontline defense mechanisms, whereby host cell suicide blocks intracellular microbial replication and exposes them for immune attack [1]. Recent advances have uncovered several programmed cell death pathways that are highly regulated to ensure proper immune responses. Besides canonical apoptosis, which initially preserves host cell integrity and is largely anti-inflammatory, macrophages can induce pyroptosis and necroptosis, which are both highly lytic and trigger proinflammatory signals (Figure 1). Pyroptosis depends on the cysteine-aspartic proteases, caspase-1 or caspase-11, which are activated by cytosolic pattern recognition receptors [1,2]. Necroptosis is regulated by the TNFR (tumor necrosis factor receptor)-associated kinases, Ripk1 and 3, and is executed by MLKL (mixed lineage kinase domain-like) [3]. Successful intracellular pathogens must suppress programmed cell death signals during the replicative or latent phase but can potentially induce these signals to promote egress and dissemination. The timing and specificity of the cell death signal can dramatically influence pathogen or host survival, suggesting a complex interplay exists between inducing potent immunity and promoting pathogen egress. Most work in this area has focused on bacteria and parasites, but more recent studies have provided exciting evidence that fungal pathogens likewise modulate host cell death pathways.

Life, Death, and Everything in between—Microbial Egress and Apoptosis

Generally, intracellular pathogens suppress apoptosis to promote replication. In addition, apoptosis can also block efficient escape, as is the case with *Mycobacterium tuberculosis* (Figure 1) [4]. Avirulent *Mycobacteria* that fail to inhibit apoptosis become trapped, as the plasma membrane remains intact for several hours after host cell death. This leads to uptake and digestion of the apoptotic cell together with *Mycobacteria* by uninfected macrophages [4]. To prevent this, *Mycobacteria* induce necroptosis, which results in plasma membrane rupture, bacterial release, and survival [5]. The ability to interfere with cell death signaling can depend on the host cell involved. For example, dendritic cells, but not macrophages, rapidly undergo apoptosis and prevent intracellular replication of *Legionella* [6]. Another example is the protozoan parasite *Leishmania*, which also blocks apoptosis in macrophages [7]. However, the first phagocytes encountered during infection are neutrophils. After phagocytosis by neutrophils, *Leishmania* manages to move into a permissive niche of macrophages by inducing apoptosis of the infected neutrophil [8]. This is partly because the "find-me/eat-me" signal displayed by

the apoptotic infected neutrophil triggers uptake of parasites and anti-inflammatory responses of macrophages that enable intracellular parasite survival [9]. Fungal pathogens also target apoptosis. Intracellular *Aspergillus* conidia block macrophage apoptosis by up-regulating prosurvival signaling, which leads to inhibition of caspase-3 activity [10]. This is thought to provide immune protection for conidia inside the phagocytes. For egress, intracellular conidia generate germ tubes and hyphae that appear to pierce through host membranes [11]. Hyphae secrete gliotoxin, which triggers intrinsic apoptosis of host immune cells by inducing mitochondrial pore formation via Bak and activation of caspase-3 [12,13], but its role during egress remains ill defined. The facultative intracellular fungus *Cryptococcus* induces host cell apoptosis, which, in contrast to *Mycobacteria*, may in this case promote escape from macrophages. Intracellular *Cryptococcus* produces large amounts of the polysaccharide-enriched capsule, which is frequently shed into discrete host vesicles [14]. At least two capsule glycans, galactoxylomannan (GalXM) and glucuronoxylomannan (GXM), have been shown to activate apoptosis in macrophages either by inducing cell surface death receptors or via intrinsic Bax/Bak-dependent apoptosis [15–18]. While the pathogenic fungus *Candida albicans* also modulates apoptotic signaling [19], there is little evidence so far that egress from macrophages relies on host cell apoptosis [20–22].

Mostly Harmless—Pathogen Escape without Host Cell Lysis

Cytosolic microbes can also egress and spread between host cells via propulsive forces due to actin polymerization, which generates protrusions at the plasma membrane [23]. These protrusions are engulfed by neighboring cells, facilitating transfer of intracellular pathogens, such as *Shigella*, without host cell death. Vacuolar pathogens can also egress without host cell lysis. Inclusions of *Chlamydia* recruit and activate the myosin motor complex and are extruded from cells via actin polymerization [24]. *Cryptococcus* is known to hijack the ability of (phago)lysosomes to fuse directly with the plasma membrane, resulting in the release of lysosomal
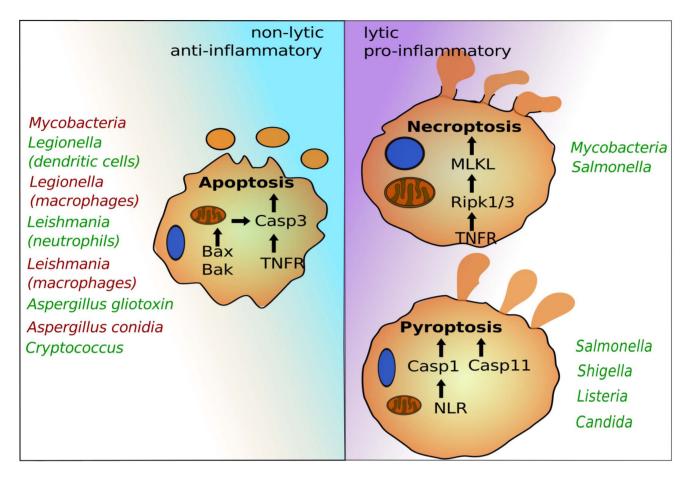
Figure 1. The programmed cell death pathways apoptosis, pyroptosis, and necroptosis play critical roles in egress of intracellular pathogens. Intrinsic apoptosis is dependent on mitochondrial damage by Bax and Bak and activation of caspase-3. Caspase-3 can also be activated via cell surface receptors such as TNFR in the pathway of extrinsic apoptosis. Pyroptosis depends on caspase-1, which is activated by cytosolic NLRs, and caspase-11, for which the precise signaling pathway needs to be determined. Necroptosis requires Ripk1 and 3 and MLKL. Representative intracellular pathogens are listed that modulate these host suicide programs, either by blocking (red) or inducing (green) their activation.
doi:10.1371/journal.ppat.1004201.g001

content including fungal cells [25,26]. This form of nonlytic exocytosis or "vomocytosis" depends on the interplay between several host factors such as phagosomal pH, inflammatory signals, host cell microtubules, actin polymerization, and exocytosis signals [27,28]. *C. albicans* has also been reported to escape from macrophages without concomitant macrophage lysis in a small fraction of cases (<1%) [29].

So Long and Thanks for All the Inflammatory Cell Death

In contrast to nonlytic escape, pathogens can also induce extensive host cell damage and death during egress. While some bacteria can escape from their vacuoles without causing concomitant host cell death [30], cytosolic bacteria, as well as microbial molecules that reach the cytosol, can be detected by NOD-like receptors (NLRs) that induce caspase-1-dependent pyroptosis (Figure 1) [1]. In addition, contamination of the cytosol by lipopolysaccharide (LPS) triggers pyroptosis via the activation of caspase-11 [31]. Others, like *Shigella*, induce caspase-1-dependent pyroptosis by triggering phagosome rupture caused by secretion of ion channels [32]. Vacuolar pathogens can also induce pyroptosis by secreting effector proteins that are recognized by NLRs into host cytosols [1,33]. Besides pyroptosis, intracellular *Salmonella*

may also activate necroptosis for escape from macrophages, at least under certain conditions [34]. Both pyroptosis and necroptosis trigger potent antimicrobial immune responses, but these types of lytic host cell death also result in rapid release of intracellular pathogens, enabling dissemination and replication in rich extracellular niches [1,34]. Very recently, intracellular *C. albicans* has been shown to trigger caspase-1-dependent pyroptosis in macrophages, the first evidence of a fungal pathogen causing pyroptosis [20,35]. This new discovery questions the long-standing view that *C. albicans* hyphal filaments kill macrophages by piercing host membranes [36]. Instead, phenotypes of several *Candida* mutants suggest that filaments contain additional features likely related to cell surface characteristics, which are necessary for induction of pyroptosis [20,37,38]. The cell wall carbohydrates, including β-glucans, are highly immunogenic because they are sensed by several macrophage lectin receptors, such as Dectin-1, that enable priming of the NLRP3 inflammasome [39,40]. There is, however, little evidence that the β-glucans can trigger pyroptosis directly, suggesting that *Candida* may depend on additional factors. Other fungal pathogens, such as *Aspergillus fumigatus*, also induce caspase-1 activation [41]. Thus, pyroptosis could be a common mechanism for fungal egress. Of note, *C. albicans* hyphae can also egress from macrophage in a pyroptosis-independent manner, particularly when fungal loads are high

[20,35]. The precise nature of the pyroptosis-independent killing of macrophages by *C. albicans* remains to be determined, but it was proposed that it could represent necrotic death when macrophages are overloaded with fungal cells [35].

And Another Thing: Egress Pathways Determine Clinical Outcome

Induction of programmed cell pathways can dramatically determine the health of the host and the pathogen, suggesting that microbial egress via host cell suicide needs to be carefully balanced. For instance, caspase-1-dependent pyroptosis triggers potent antimicrobial responses targeted at escaped *Salmonella* [1], while caspase-11-dependent pyroptosis (in the absence of caspase-1 activation) actually promotes extracellular bacterial replication and dissemination to the detriment of the host [42]. Similarly, caspase-1-dependent immune response can trigger antifungal inflammation [40], but "hijacking" macrophage pyroptosis promotes rapid egress of *C. albicans* from macrophages [20,35]. Not all proinflammatory responses result in clearance of intracellular pathogens. For example, *Salmonella* can utilize type 1 interferon signaling for egress from macrophages and for survival in mice [34,42]. Furthermore, some microbes thrive under proinflammatory conditions, including *Listeria* and some *Leishmania* species, suggesting that the timing and manner of programmed cell death is critical for pathogen egress and disease [43–45]. In the case of tuberculosis, the level of inflammatory responses determines macrophage cell death signaling, disease progression, and pathogen clearance [5]. Finally, pathogen-provoked host cell death itself can contribute to disease outcome, as LPS-induced septic shock results from uncontrolled caspase-11-dependent pyroptosis [2]. Similarly, while caspase-1-dependent secretion of the proinflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18) has potent antifungal properties, symptoms of vaginal candidiasis are largely caused by sustained influx of neutrophils due to IL-1β secretion [46]. Given that immunopathology is also associated with fatal candidiasis [47], the fungal egress pathway in macrophages may directly contribute to disease progression.

References

1. Miao EA, Leaf IA, Treuting PM, Mao DP, Dors M, et al. (2010) Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. Nat Immunol 11: 1136–1142.
2. Kayagaki N, Warming S, Lamkanfi M, Vande Walle L, Louie S, et al. (2011) Non-canonical inflammasome activation targets caspase-11. Nature 479: 117–121.
3. Sun L, Wang H, Wang Z, He S, Chen S, et al. (2012) Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. Cell 148: 213–227.
4. Martin CJ, Booty MG, Rosebrock TR, Nunes-Alves C, Desjardins DM, et al. (2012) Efferocytosis is an innate antibacterial mechanism. Cell Host Microbe 12: 289–300.
5. Roca FJ, Ramakrishnan L (2013) TNF dually mediates resistance and susceptibility to mycobacteria via mitochondrial reactive oxygen species. Cell 153: 521–534.
6. Nogueira CV, Lindsten T, Jamieson AM, Case CL, Shin S, et al. (2009) Rapid pathogen-induced apoptosis: a mechanism used by dendritic cells to limit intracellular replication of *Legionella pneumophila*. PLoS Pathog 5: e1000478.
7. Srivastav S, Basu Ball W, Gupta P, Giri J, Ukil A, et al. (2014) *Leishmania donovani* prevents oxidative burst-mediated apoptosis of host macrophages through selective induction of suppressors of cytokine signaling (SOCS) proteins. J Biol Chem 289: 1092–1105.
8. van Zandbergen G, Klinger M, Mueller A, Dannenberg S, Gebert A, et al. (2004) Cutting edge: neutrophil granulocyte serves as a vector for *Leishmania* entry into macrophages. J Immunol 173: 6521–6525.
9. Peters NC, Egen JG, Secundino N, Debrabant A, Kimblin N, et al. (2008) *In vivo* imaging reveals an essential role for neutrophils in leishmaniasis transmitted by sand flies. Science 321: 970–974.
10. Volling K, Thywissen A, Brakhage AA, Saluz HP (2011) Phagocytosis of melanized Aspergillus conidia by macrophages exerts cytoprotective effects by sustained PI3K/Akt signalling. Cell Microbiol 13: 1130–1148.
11. Wasylnka JA, Moore MM (2002) Uptake of *Aspergillus fumigatus* Conidia by phagocytic and nonphagocytic cells in vitro: quantitation using strains expressing green fluorescent protein. Infect Immun 70: 3156–3163.
12. Stanzani M, Orciuolo E, Lewis R, Kontoyiannis DP, Martins SL, et al. (2005) *Aspergillus fumigatus* suppresses the human cellular immune response via gliotoxin-mediated apoptosis of monocytes. Blood 105: 2258–2265.
13. Geissler A, Haun F, Frank DO, Wieland K, Simon MM, et al. (2013) Apoptosis induced by the fungal pathogen gliotoxin requires a triple phosphorylation of Bim by JNK. Cell Death Differ 20: 1317–1329.
14. Tucker SC, Casadevall A (2002) Replication of *Cryptococcus neoformans* in macrophages is accompanied by phagosomal permeabilization and accumulation of vesicles containing polysaccharide in the cytoplasm. Proc Natl Acad Sci U S A 99: 3165–3170.
15. Villena SN, Pinheiro RO, Pinheiro CS, Nunes MP, Takiya CM, et al. (2008) Capsular polysaccharides galactoxylomannan and glucuronoxylomannan from *Cryptococcus neoformans* induce macrophage apoptosis mediated by Fas ligand. Cell Microbiol 10: 1274–1285.
16. Monari C, Paganelli F, Bistoni F, Kozel TR, Vecchiarelli A (2008) Capsular polysaccharide induction of apoptosis by intrinsic and extrinsic mechanisms. Cell Microbiol 10: 2129–2137.
17. De Jesus M, Nicola AM, Frases S, Lee IR, Mieses S, et al. (2009) Galactoxylomannan-mediated immunological paralysis results from specific B cell depletion in the context of widespread immune system damage. J Immunol 183: 3885–3894.
18. Ben-Abdallah M, Sturny-Leclere A, Ave P, Louise A, Moyrand F, et al. (2012) Fungal-induced cell cycle impairment, chromosome instability and apoptosis via differential activation of NF-kappaB. PLoS Pathog 8: e1002555.
19. Ibata-Ombetta S, Idziorek T, Trinel PA, Poulain D, Jouault T (2003) *Candida albicans* phospholipomannan promotes survival of phagocytosed yeasts through modulation of bad phosphorylation and macrophage apoptosis. J Biol Chem 278: 13086–13093.
20. Uwamahoro N, Verma-Gaur J, Shen HH, Qu Y, Lewis R, et al. (2014) The Pathogen *Candida albicans* Hijacks Pyroptosis for Escape from Macrophages. MBio 5: e00003-14.
21. Reales-Calderon JA, Sylvester M, Strijbis K, Jensen ON, Nombela C, et al. (2013) *Candida albicans* induces pro-inflammatory and anti-apoptotic signals in macrophages as revealed by quantitative proteomics and phosphoproteomics. J Proteomics 91C: 106–135.
22. Lionakis MS, Swamydas M, Fischer BG, Plantinga TS, Johnson MD, et al. (2013) CX3CR1-dependent renal macrophage survival promotes *Candida* control and host survival. J Clin Invest 123: 5035–5051.
23. Fukumatsu M, Ogawa M, Arakawa S, Suzuki M, Nakayama K, et al. (2012) *Shigella* targets epithelial tricellular junctions and uses a noncanonical clathrin-dependent endocytic pathway to spread between cells. Cell Host Microbe 11: 325–336.
24. Lutter EI, Barger AC, Nair V, Hackstadt T (2013) *Chlamydia trachomatis* inclusion membrane protein CT228 recruits elements of the myosin phosphatase pathway to regulate release mechanisms. Cell Rep 3: 1921–1931.
25. Alvarez M, Casadevall A (2006) Phagosome extrusion and host-cell survival after *Cryptococcus neoformans* phagocytosis by macrophages. Curr Biol 16: 2161–2165.
26. Johnston SA, May RC (2010) The human fungal pathogen Cryptococcus neoformans escapes macrophages by a phagosome emptying mechanism that is inhibited by Arp2/3 complex-mediated actin polymerisation. PLoS Pathog 6: e1001041.
27. Johnston SA, May RC (2013) *Cryptococcus* interactions with macrophages: evasion and manipulation of the phagosome by a fungal pathogen. Cell Microbiol 15: 403–411.
28. Nicola AM, Robertson EJ, Albuquerque P, Derengowski LdS, Casadevall A (2011) Nonlytic exocytosis of *Cryptococcus neoformans* from macrophages occurs in vivo and is influenced by phagosomal pH. MBio 2: e00167-11.
29. Bain JM, Lewis LE, Okai B, Quinn J, Gow NA, et al. (2012) Non-lytic expulsion/exocytosis of *Candida albicans* from macrophages. Fungal Genet Biol 49: 677–678.
30. Sauer JD, Pereyre S, Archer KA, Burke TP, Hanson B, et al. (2011) *Listeria monocytogenes* engineered to activate the Nlrc4 inflammasome are severely attenuated and are poor inducers of protective immunity. Proc Natl Acad Sci U S A 108: 12419–12424.
31. Aachoui Y, Leaf IA, Hagar JA, Fontana MF, Campos CG, et al. (2013) Caspase-11 protects against bacteria that escape the vacuole. Science 339: 975–978.
32. Senerovic L, Tsunoda SP, Goosmann C, Brinkmann V, Zychlinsky A, et al. (2012) Spontaneous formation of IpaB ion channels in host cell membranes reveals how *Shigella* induces pyroptosis in macrophages. Cell Death Dis 3: e384.
33. Casson CN, Copenhaver AM, Zwack EE, Nguyen HT, Strowig T, et al. (2013) Caspase-11 Activation in Response to Bacterial Secretion Systems that Access the Host Cytosol. PLoS Pathog 9: e1003400.
34. Robinson N, McComb S, Mulligan R, Dudani R, Krishnan L, et al. (2012) Type I interferon induces necroptosis in macrophages during infection with *Salmonella enterica serovar* Typhimurium. Nat Immunol 13: 954–962.

35. Wellington M, Koselny K, Sutterwala FS, Krysan DJ (2014) *Candida albicans* triggers NLRP3-mediated pyroptosis in macrophages. Eukaryot Cell 13: 329–340.
36. Lo HJ, Kohler JR, DiDomenico B, Loebenberg D, Cacciapuoti A, et al. (1997) Nonfilamentous C. albicans mutants are avirulent. Cell 90: 939–949.
37. Wellington M, Koselny K, Krysan DJ (2012) *Candida albicans* Morphogenesis Is Not Required for Macrophage Interleukin 1beta Production. MBio 4: e00433-12.
38. Lowman DW, Greene RR, Bearden DW, Kruppa MD, Pottier M, et al. (2013) Novel structural features in *Candida albicans* hyphal glucan provide a basis for differential innate immune recognition of hyphae versus yeast. J Biol Chem 289: 3432–3443.
39. Kankkunen P, Teirila L, Rintahaka J, Alenius H, Wolff H, et al. (2010) (1,3)-beta-glucans activate both dectin-1 and NLRP3 inflammasome in human macrophages. J Immunol 184: 6335–6342.
40. Gross O, Poeck H, Bscheider M, Dostert C, Hannesschlager N, et al. (2009) Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence. Nature 459: 433–436.
41. Said-Sadier N, Padilla E, Langsley G, Ojcius DM (2010) *Aspergillus fumigatus* stimulates the NLRP3 inflammasome through a pathway requiring ROS production and the Syk tyrosine kinase. PLoS ONE 5: e10008.
42. Broz P, Ruby T, Belhocine K, Bouley DM, Kayagaki N, et al. (2012) Caspase-11 increases susceptibility to *Salmonella* infection in the absence of caspase-1. Nature 490: 288–291.
43. Auerbuch V, Brockstedt DG, Meyer-Morse N, O'Riordan M, Portnoy DA (2004) Mice lacking the type I interferon receptor are resistant to *Listeria monocytogenes*. J Exp Med 200: 527–533.
44. O'Connell RM, Saha SK, Vaidya SA, Bruhn KW, Miranda GA, et al. (2004) Type I interferon production enhances susceptibility to *Listeria monocytogenes* infection. J Exp Med 200: 437–445.
45. Xin L, Vargas-Inchaustegui DA, Raimer SS, Kelly BC, Hu J, et al. (2010) Type I IFN receptor regulates neutrophil functions and innate immunity to *Leishmania* parasites. J Immunol 184: 7047–7056.
46. Peters BM, Palmer GE, Fidel PL, Jr., Noverr MC (2013) Fungal morphogenetic pathways are required for the hallmark inflammatory response during Candida vaginitis. Infect Immun 82: 532–543.
47. Majer O, Bourgeois C, Zwolanek F, Lassnig C, Kerjaschki D, et al. (2012) Type I interferons promote fatal immunopathology by regulating inflammatory monocytes and neutrophils during *Candida* infections. PLoS Pathog 8: e1002811.