MyD88 in *Mycobacterium tuberculosis* infection

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Abstract MyD88 adaptor protein mediates numerous biologically important signal transduction pathways in innate immunity. MyD88 signaling fosters bacterial containment and is necessary to raise an adequate innate and acquired immune response to *Mycobacterium tuberculosis* (Mtb). The phagosome is a crucial cellular location not only for Mtb replication, but it is also where components of the Myddosome and inflammasome are recruited. Besides its function as a TLR-adaptor protein, MyD88 may help stabilizing cytosolic receptors that are recruited to the phagosome. MyD88 plays a critical role not only in the generation of an inflammatory response, but also in inducing regulatory signals to prevent excessive inflammation and cellular damage in the lung.

Keywords *Mycobacterium tuberculosis* · MyD88 · Phagosome · Regulation of inflammation

Introduction

Toll-like receptors (TLRs) are transmembrane receptors which recognize various pathogen-associated molecular patterns (PAMPs) [1]. The myeloid differentiation primary response gene 88 (MyD88) is critical for TLR signaling [2], and except for TLR3, all TLRs require MyD88 for their downstream signaling [1, 3]. First identified as a myeloid differentiation gene [4], MyD88 was later on shown to

be an essential adaptor protein in the signaling pathway, required for NF- κ B activation in response to IL-1R1 signaling [5–7]. MyD88 adaptor protein mediates numerous biologically important signal transduction pathways in innate immunity [8]. Also, MyD88 signaling is important in the regulation of inflammation during bacterial infection and cancer progression [9].

MyD88 mutations are associated with immunodeficiencies that predispose patients to recurrent life-threatening bacterial infections, similar to what is observed in MyD88-deficient mice which are susceptible to various pathogens [10, 11]. MyD88-deficient macrophages are able to express proinflammatory cytokines, but are defective in TNF, IL-12, and NO production in response to mycobacterial stimulation [12]. They are also able to expand IFN- γ -producing antigen-specific T cells, but in a delayed fashion [13]. Infection with Mtb in these mice is lethal, as MyD88 signaling is necessary to raise an adequate innate and acquired immune response to Mtb [12–14]. The functions of MyD88 appear to go beyond its signaling pathway, as we will be describing in the following sections.

MyD88 in TB phagocytosis

The phagocytic process starts with recognition of the pathogen. This occurs through receptors present on the cell surface, such as scavenger or mannose receptors, which bind ligands present on the surface of the pathogen [15]. Upon ligation, these receptors cluster on the phagocyte surface and induce rearrangement of the actin cytoskeleton. TLR stimulation upregulates the Fc receptor, complement receptor, scavenger receptors MARCO and SR-A, and many other genes regulating phagocytosis. Upregulation of these genes is dependent on p38 activation, downstream of

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MyD88-TLR signaling [16]. Mtb interacts with MARCO, which tethers cell wall glycolipid, trehalose 6,6'-dimycolate (TDM/cord factor) from Mtb and activates a TLR2/CD14 signaling pathway [17]. CD36 and MARCO can interact with TLR2 and CD14, regulating NF- κ -B cytokine response, and are all required for macrophage cytokine responses to mycobacterial TDM and Mtb [17] (Fig. 1a).

Many genes involved in the subsequent actin rearrangement that occurs during the phagocytosis process are dependent on MyD88 [18], so it is no surprise that phagocytosis of bacteria is impaired in the absence of MyD88 [19].

Phagocytosis, in turn, is essential for an optimal MyD88-dependent response to bacteria [20]. In addition to playing a central role in phagocytosis, MyD88 is crucial to the downstream TLR activation and signaling occurring after bacterial degradation in the phagosome of human monocytes [21] and murine macrophages [20]. Bacteria must be phagocytized and it is within the acidic environment of

the phagosome that activated host enzymes and endosomal TLR receptors can recognize their bacterial ligands. Once these bacterial-derived ligands have been liberated after internalization and degradation, a TLR-dependent response can be initiated from the phagosome [20, 22, 23].

Besides being necessary for mounting an adequate innate and adaptive immune response [12, 14], MyD88 signaling in DCs and macrophages is essential to control the mycobacterial burden and prevent pathogen persistence [14, 24]. In the case of Mtb phagocytosis, MyD88 is necessary for confinement of Mtb in the phagosome [25].

PRR recognition of Mtb

Several pattern recognition receptors (PRRs) on phagocytic cells have been identified in the recognition of conserved (PAMPs) of mycobacteria [26]. Mtb can mask macrophage PRRs via cell surface-associated phthiocerol

Fig. 1 The distinct roles of MyD88 in Mtb infection. **a** PRR-mediated phagocytosis of Mtb. Scavenger receptors like MARCO interact with TLR2 and CD14. MARCO tethers the cell wall from Mtb and activates a TLR2/CD14 signaling pathway. Upregulation of scavenger receptors is in turn dependent on activation of downstream MyD88-TLR signaling. **b** Phagosomal presence of Mtb and/or Mtb structural components liberated in the phagosome lead to PRR activation. TLR activation by Mtb ligands leads to downstream NF- κ B-

signaling and IRF-mediated responses. **c** Expression of inflammatory and anti-inflammatory cytokines ensues. **d** MyD88 also regulates the expression of various negative regulators (e.g., IRAK-M, SOCS-1 and SOCS-3, etc.) in addition to IL-10 and type I IFNs **e** which suppress host protective inflammatory mediators. **f** The Myddosome may also have a functional role in stabilizing “cytosolic” receptors when recruited to the phagosome

dimycocerosate (PDIM) lipids. These lipids also promote the recruitment of Mtb-permissive macrophages, while impairing reactive oxygen species (ROS)-response of microbicidal macrophages [27].

Mtb is a bacterium that mainly resides in the phagosome [28], and has evolved mechanisms to evade its destruction in phagolysosomes to successfully survive and replicates within phagocytes [29]. Suboptimal recognition by phagosomal receptors seems important in deciding the fate of this organism and active disease in the host. This could explain the association of different endosomal TLR polymorphisms associated with pulmonary TB in different populations [30–33].

Various pathogenic mycobacterial species might be able to invade the cytosol as it has been able to previously circumvent phagosomal maturation [34]. This process of phagosomal escape may occur at later stages of infection [35], and appears to rely on the region of deletion 1 (RD1)-dependent secretion of early secreted antigenic target 6 (ESAT-6) [36], otherwise reported to possess pore-forming activity [37]. Virulent strains of Mtb reported to translocate from the phagosome into the cytosol of macrophages and dendritic cells (DC) [25, 36] must inhibit innate immune signals to facilitate their translocation to the cytosol. This is accomplished by down-regulation of key genes in the TLR2-MyD88 pathway [25].

Some studies proposing that Mtb might be able to escape into the cytosol of macrophages, alluding to the inflammasome activation and cell death after infection by Mtb and other mycobacteria [38, 39], do not clarify if these events are due to phagosomal escape or a consequence of phagosomal membrane rupture which has previously been found to occur [40]. Furthermore, rupture of the phagosomal membrane by Mtb results in necrotic cell death of the infected macrophages [41].

Cytosolic receptor activation by recognition of their respective Mtb ligand may not necessarily require cytosolic escape of the bacillus. Intraphagosomal Mtb, is able to perforate the phagosomal membrane, allowing for the transfer of phagosomal contents in to the cytosol [42, 43]. NLRP3 is activated after recognition of Mtb's ESAT-6 [44]. ESAT-6 is known to possess pore-forming activity, and in fact, NLRP3 activation and necrosis are dependent on ESAT-6-damage to the phagosomal membrane [38, 41].

NOD2, a member of NOD-like receptors, recognizes mycobacterial N-glycolyl muramyl dipeptide (MDP) within the cytoplasm [45], and along with MyD88, is also required for optimal inflammatory response and protective immunity to Mtb.

DC-SIGN and dectin-1, both members of C-type lectin receptors, are able to selectively recognize mannose caps on lipoarabinomannan as well as β -glucan present in Mycobacteria [46, 47]. Mtb-induced DC-SIGN signaling affects

TLR signaling at the transcriptional level [48]. In summary, Mtb-derived ligands are being sensed via endosomal receptors or recruitment of cytosolic receptors to the phagosome, or by cytosolic transfer due to loss of phagosomal integrity.

The Myddosome and cytosolic PRR phagosomal recruitment

Although MyD88 appears to be essential for an effective innate immune response against Mtb [14, 49], controversy exists if TLRs may or may not be essential. These discrepancies may be resolved if we consider the distinct immune functions of the TLR/MyD88 system in TB, beyond the function of MyD88 as a TLR adaptor. The Myddosome is a multiprotein complex involving MyD88 that forms after TLR activation [50, 51]. Myddosome stabilizes the weakly associated receptor–adaptor Toll-IL-1 receptor resistance (TIR) complexes [51], and could serve stabilizing NOD2 and NLRP3 when these “cytosolic” receptors are recruited to the phagosome [52, 53]. This could help explain macrophage activation observed in response to non-pathogenic mycobacteria through TLR2 and Nod2 occurring in an MyD88-dependent manner [54] or the synergistic inflammatory response by these two receptors [55].

Mtb possesses a system to prevent inflammasome [56] and IL-1 β activation [57]. Although NLRP3 activation by Mtb does exist [58], NALP3 does not appear to be necessary for protection against Mtb [59]. NLRP3 activation could occur at the phagosomal level, a phenomenon described in *Staphylococcus aureus* infection [60]. All this suggests an interaction of MyD88 with other “cytosolic” receptors at the phagosome, where the MyD88 could exert a regulatory function on other pathways like that of the inflammasome.

MyD88 in the inflammatory response to Mtb

The importance of MyD88 in the protection against Mtb infection is underlined by the fact that MyD88 polymorphisms have been associated with pulmonary TB [61]. Early post-infection, Mtb elicits inflammation-dampening signals like NF- κ B dampening molecule TNFAIP3 (A20) in macrophages which would favor its survival [62]. ESAT-6 can down regulate MyD88-dependent TLR signaling [63]. Proteins like Rv0625, which are heavily secreted by virulent strains like the Beijing strain can induce a strong TNF and MCP-1 response which occurs in an MyD88-dependent manner [64]. Mycobacterial TLR-signaling crosstalks with other intracellular antimicrobial innate pathways, like the autophagy process and functional vitamin D receptor (VDR) signaling [62]. Infection with Mtb

induces differential expression (DE) of a series of genes in MyD88-independent pathways (i.e., iNOS, COX-2, IP10, MIG, RANTES, IRG-1, argininosuccinate synthetase 1, and chemokines JE and KC) [65].

Macrophages in the lung are the primary cells encountering Mtb, and along with neutrophils, they are crucial in mounting an early innate immune response. Macrophages can be activated by cytokines and microbial products [66]. MyD88 primes macrophages for IFN- γ mediated activation [65]. In fact full-scale activation of macrophages by IFN- γ can only be achieved when MyD88 is present. Mtb inhibits macrophage responses to IFN- γ through MyD8-dependent and MyD88-independent mechanisms [67]. When MyD88 k.o. mice are infected with Mtb, rather than being highly susceptible to the infection they develop granulomatous pulmonary lesions with larger neutrophil infiltration compared to wild-type (WT) mice [68]. These massive cellular infiltrations accompanied by extensive necrosis of granulomas are also described in a later study that reported high susceptibility of MyD88 k.o. mice to Mtb infection [13]. Both studies are also concordant in a higher bacterial burden in the lungs and spleens of MyD88 k.o. mice at 5 weeks post-infection [13, 68]. Lifespan of neutrophils is short, and removal of apoptotic cells by resident macrophages is key in the resolution of inflammation. Dying neutrophils also enhance the capacity of infected macrophages to control intracellular growth of Mtb [69]. NLRP3 inflammasome activation and IL-1 β signaling by neutrophils, however, appear to enhance macrophage proinflammatory activation. IL-1R-mediated signaling is MyD88-dependent and is crucial in the early control of Mtb infection [70]. IL-1 and type I IFNs represent two major counter-regulatory mechanisms that control the outcome of Mtb infection [71]. IL-1 confers host resistance through the induction of eicosanoids (like PGE2) that limit excessive type I interferon (IFN) production keeping bacteria growth in check [71, 72]. Type I IFNs promote disease by inducing IL-10 and IL1 receptor antagonist [73]. The type I interferon, IFN- β , is induced after Mtb infection and can also suppress NLRP3-inflammasome activation [74]. All this evidence connects Mtb phagosomal recognition and phagocyte MyD88-regulated signaling, with subsequent neutrophil recruitment, that ultimately is associated with pulmonary disease [75].

“Losing control” in the absence of MyD88

Progressive cellular destruction in the lung is associated with massive neutrophilic infiltration suggesting that inflammation may be a key factor in progression towards active tuberculosis [76]. A down-regulation of the NF- κ B response is a necessary mechanism to prevent excessive inflammation and return to homeostasis [77]. MyD88s acts

as a negative signaling factor to shut down LPS-induced NF- κ B activation [78]. Regulation of the inflammatory response by MyD88 might be an indirect process (e.g., through MyD88-dependent expression of long non-coding RNA lincRNA-Cox2) [79]. MyD88s-mediated inhibition of TLR signaling is selective for the NF- κ B pathway and does not alter the activity of other pathways, like the one of transcription factor AP-1 [80]. Resolution of inflammation also involves apoptosis and subsequent clearance of activated inflammatory cells, in order to prevent a chronic inflammatory condition [81]. An aberrant inflammatory response ensues when MyD88 is absent, possibly due to a failure in the mechanism of regulation of inflammation. Mice lacking MyD88 are hyper-susceptible to dextran sodium sulfate (DSS)-induced colitis [82–84], and present an increased and more persistent inflammation after *Borrelia burgdorferi* infection [85–87]. In fact, in vivo infection of Mtb has shown higher levels of proinflammatory cytokines in lung tissue and sera of MyD88 k.o. mice [68]. Absence of MyD88 can lead to a Th10-mediated autoimmunity when mice are immunized with complete Freund’s adjuvant [88], composed of heat killed and dried avirulent Mtb strain H37Ra.

MicroRNAs (miRNAs) are important regulators of TLR signaling. miR-149 regulates MyD88 expression in murine macrophages [89]. In alveolar epithelial cells, immunomodulatory miR-124 is induced upon infection with BCG in an MyD88-dependent manner [90]. Although miR-124 and miR-147 target MyD88 and TRAF6, and are highly induced by Mtb to promote Mtb growth [91], they may also reflect an MyD88 mechanism of regulation of inflammation.

BCG, the attenuated *M. bovis* strain bacilli Calmette–Guerin, is the only currently used vaccine that has variable protective efficacy against Mtb infection [92]. Future vaccine models utilizing recombinant non-pathogenic *M. smegmatis* bearing Mtb genes (IKEPLUS strain) appear to be able to induce bactericidal immunity against challenge with virulent Mtb [93]. This response can only occur in the presence of MyD88, since infection with this recombinant *M. smegmatis* is lethal in MyD88-deficient mice [93]. The functions of MyD88 in Mtb infection are multiple and should be taken into account when assessing an individual’s genetic risk, severe disease predisposition, and designing strategies to control Mtb infection.

References

1. Kawai T, Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 11:373–384

2. Medzhitov R, Preston-Hurlburt P, Janeway CA Jr (1997) A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* 388:394–397
3. Kawai T, Adachi O, Ogawa T, Takeda K, Akira S (1999) Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity* 11:115–122
4. Lord KA, Hoffman-Liebermann B, Liebermann DA (1990) Nucleotide sequence and expression of a cDNA encoding MyD88, a novel myeloid differentiation primary response gene induced by IL6. *Oncogene* 5:1095–1097
5. Muzio M, Ni J, Feng P, Dixit VM (1997) IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science* 278:1612–1615
6. Wesche H, Henzel WJ, Shillinglaw W, Li S, Cao Z (1997) MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. *Immunity* 7:837–847
7. Hultmark D (1994) Macrophage differentiation marker MyD88 is a member of the Toll/IL-1 receptor family. *Biochem Biophys Res Commun* 199:144–146
8. Warner N, Nunez G (2013) MyD88: a critical adaptor protein in innate immunity signal transduction. *J Immunol* 190:3–4
9. Wang JQ, Jeelall YS, Ferguson LL, Horikawa K (2014) Toll-Like Receptors and Cancer: MYD88 Mutation and Inflammation. *Front Immunol* 5:367
10. von Bernuth H, Picard C, Puel A, Casanova JL (2012) Experimental and natural infections in MyD88- and IRAK-4-deficient mice and humans. *Eur J Immunol* 42:3126–3135
11. von Bernuth H, Picard C, Jin Z, Pankla R, Xiao H, Ku CL, Chrabieh M, Mustapha IB, Ghandil P, Camcioglu Y, Vasconcelos J, Sirvent N, Guedes M, Vitor AB, Herrero-Mata MJ, Arostegui JJ, Rodrigo C, Alsina L, Ruiz-Ortiz E, Juan M, Fortuny C, Yague J, Anton J, Pascal M, Chang HH, Janniere L, Rose Y, Garty BZ, Chapel H, Issekutz A, Marodi L, Rodriguez-Gallego C, Banchereau J, Abel L, Li X, Chaussabel D, Puel A, Casanova JL (2008) Pyogenic bacterial infections in humans with MyD88 deficiency. *Science* 321:691–696
12. Fremont CM, Yermeev V, Nicolle DM, Jacobs M, Quesniaux VF, Ryffel B (2004) Fatal *Mycobacterium tuberculosis* infection despite adaptive immune response in the absence of MyD88. *J Clin Invest* 114:1790–1799
13. Holscher C, Reiling N, Schaible UE, Holscher A, Bathmann C, Korb D, Lenz I, Sonntag T, Kroger S, Akira S, Mossmann H, Kirschning CJ, Wagner H, Freudenberg M, Ehlers S (2008) Containment of aerogenic *Mycobacterium tuberculosis* infection in mice does not require MyD88 adaptor function for TLR2, -4 and -9. *Eur J Immunol* 38:680–694
14. Berod L, Stuve P, Swallow M, Arnold-Schrauf C, Kruse F, Gentilini MV, Freitag J, Holzmann B, Sparwasser T (2014) MyD88 signalling in myeloid cells is sufficient to prevent chronic mycobacterial infection. *Eur J Immunol* 44:1399–1409
15. Tricker E, Cheng G (2008) With a little help from my friends: modulation of phagocytosis through TLR activation. *Cell Res* 18:711–712
16. Blander JM, Medzhitov R (2004) Regulation of phagosome maturation by signals from toll-like receptors. *Science* 304:1014–1018
17. Bowdish DM, Sakamoto K, Kim MJ, Kroos M, Mukhopadhyay S, Leifer CA, Tryggvason K, Gordon S, Russell DG (2009) MARCO, TLR2, and CD14 are required for macrophage cytokine responses to mycobacterial trehalose dimycolate and *Mycobacterium tuberculosis*. *PLoS Pathog* 5:e1000474
18. Doyle SE, O'Connell RM, Miranda GA, Vaidya SA, Chow EK, Liu PT, Suzuki S, Suzuki N, Modlin RL, Yeh WC, Lane TF, Cheng G (2004) Toll-like receptors induce a phagocytic gene program through p38. *J Exp Med* 199:81–90
19. Shin OS, Miller LS, Modlin RL, Akira S, Uematsu S, Hu LT (2009) Downstream signals for MyD88-mediated phagocytosis of *Borrelia burgdorferi* can be initiated by TRIF and are dependent on PI3K. *J Immunol* 183:491–498
20. Ip WK, Sokolovska A, Charriere GM, Boyer L, Dejardin S, Cappillino MP, Yantosca LM, Takahashi K, Moore KJ, Lacy-Hulbert A, Stuart LM (2010) Phagocytosis and phagosome acidification are required for pathogen processing and MyD88-dependent responses to *Staphylococcus aureus*. *J Immunol* 184:7071–7081
21. Cervantes JL, La Vake CJ, Weinerman B, Luu S, O'Connell C, Verardi PH, Salazar JC (2013) Human TLR8 is activated upon recognition of *Borrelia burgdorferi* RNA in the phagosome of human monocytes. *J Leukoc Biol* 94:1231–1241
22. Cervantes JL, Hawley KL, Benjamin SJ, Weinerman B, Luu SM, Salazar JC (2014) Phagosomal TLR signaling upon *Borrelia burgdorferi* infection. *Front Cell Infect Microbiol* 4:55
23. Kagan JC, Iwasaki A (2012) Phagosome as the organelle linking innate and adaptive immunity. *Traffic* 13:1053–1061
24. Scanga CA, Bafica A, Feng CG, Cheever AW, Hieny S, Sher A (2004) MyD88-deficient mice display a profound loss in resistance to *Mycobacterium tuberculosis* associated with partially impaired Th1 cytokine and nitric oxide synthase 2 expression. *Infect Immun* 72:2400–2404
25. Rahman A, Sobia P, Gupta N, Kaer LV, Das G (2014) *Mycobacterium tuberculosis* subverts the TLR-2-MyD88 pathway to facilitate its translocation into the cytosol. *PLoS One* 9:e86886
26. Hossain MM, Norazmi MN: Pattern recognition receptors and cytokines in *Mycobacterium tuberculosis* infection—the double-edged sword? *BioMed research international* 2013;2013:179174.
27. Cambier CJ, Falkow S, Ramakrishnan L (2014) Host evasion and exploitation schemes of *Mycobacterium tuberculosis*. *Cell* 159:1497–1509
28. Guirado E, Schlesinger LS, Kaplan G (2013) Macrophages in tuberculosis: friend or foe. *Semin Immunopathol* 35:563–583
29. Russell D. PG, Ownes R, Rohde K, Yates R (2005) *Mycobacterium tuberculosis* and the Four-Minute Phagosome. *ASM News* 71:459–463
30. Sun Q, Zhang Q, Xiao HP, Bai C (2015) Toll-like receptor polymorphisms and tuberculosis susceptibility: A comprehensive meta-analysis. *J Huazhong Univ Sci Technol Med Sci* 35:157–168
31. Schurz H, Daya M, Moller M, Hoal EG, Salie M (2015) TLR1, 2, 4, 6 and 9 Variants Associated with Tuberculosis Susceptibility: A Systematic Review and Meta-Analysis. *PLoS One* 10:e0139711
32. Zhao L, Liu K, Kong X, Tao Z, Wang Y, Liu Y (2015) Association of polymorphisms in Toll-like receptors 4 and 9 with risk of pulmonary tuberculosis: a meta-analysis. *Med Sci Monit* 21:1097–1106
33. Chen Z, Wang W, Liang J, Wang J, Feng S, Zhang G (2015) Association between toll-like receptors 9 (TLR9) gene polymorphism and risk of pulmonary tuberculosis: meta-analysis. *BMC Pulm Med* 15:57
34. Soldati T, Neyrolles O (2012) *Mycobacteria* and the intraphagosomal environment: take it with a pinch of salt(s)! *Traffic* 13:1042–1052
35. Simeone R, Sayes F, Song O, Groschel MI, Brodin P, Brosch R, Majlessi L (2015) Cytosolic access of *Mycobacterium tuberculosis*: critical impact of phagosomal acidification control and demonstration of occurrence in vivo. *PLoS Pathog* 11:e1004650
36. van der Wel N, Hava D, Houben D, Fluittsma D, van Zon M, Pierson J, Brenner M, Peters PJ (2007) *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells. *Cell* 129:1287–1298
37. Smith J, Manoranjan J, Pan M, Bohsali A, Xu J, Liu J, McDonald KL, Szyk A, LaRonde-LeBlanc N, Gao LY (2008) Evidence

- for pore formation in host cell membranes by ESX-1-secreted ESAT-6 and its role in *Mycobacterium marinum* escape from the vacuole. *Infect Immun* 76:5478–5487
38. Wong KW, Jacobs WR Jr (2011) Critical role for NLRP3 in necrotic death triggered by *Mycobacterium tuberculosis*. *Cell Microbiol* 13:1371–1384
 39. Welin A, Raffetseder J, Eklund D, Stendahl O, Lerm M (2011) Importance of phagosomal functionality for growth restriction of *Mycobacterium tuberculosis* in primary human macrophages. *J Innate Immun* 3:508–518
 40. Teitelbaum R, Cammer M, Maitland ML, Freitag NE, Condeelis J, Bloom BR (1999) *Mycobacterial* infection of macrophages results in membrane-permeable phagosomes. *Proc Natl Acad Sci USA* 96:15190–15195
 41. Simeone R, Bobard A, Lippmann J, Bitter W, Majlessi L, Brosch R, Enninga J (2012) Phagosomal rupture by *Mycobacterium tuberculosis* results in toxicity and host cell death. *PLoS Pathog* 8:e1002507
 42. Manzanillo PS, Shiloh MU, Portnoy DA, Cox JS (2012) *Mycobacterium tuberculosis* activates the DNA-dependent cytosolic surveillance pathway within macrophages. *Cell Host Microbe* 11:469–480
 43. Watson RO, Manzanillo PS, Cox JS (2012) Extracellular *M. tuberculosis* DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. *Cell* 150:803–815
 44. Kupz A, Zedler U, Staber M, Perdomo C, Dorhoi A, Brosch R, Kaufmann SH (2016) ESAT-6-dependent cytosolic pattern recognition drives noncognate tuberculosis control in vivo. *J Clin Invest* 126:2109–2122
 45. Saiga H, Shimada Y, Takeda K (2011) Innate immune effectors in mycobacterial infection. *Clin Dev Immunol* 2011:347594
 46. Kleinnijenhuis J, Oosting M, Joosten LA, Netea MG, Van Crevel R (2011) Innate immune recognition of *Mycobacterium tuberculosis*. *Clin Dev Immunol* 2011:405310
 47. Maeda N, Nigou J, Herrmann JL, Jackson M, Amara A, Lagrange PH, Puzo G, Gicquel B, Neyrolles O (2003) The cell surface receptor DC-SIGN discriminates between *Mycobacterium* species through selective recognition of the mannose caps on lipoarabinomannan. *J Biol Chem* 278:5513–5516
 48. Gringhuis SI, den Dunnen J, Litjens M, van der Vlist M, Geijtenbeek TB (2009) Carbohydrate-specific signaling through the DC-SIGN signalosome tailors immunity to *Mycobacterium tuberculosis*, HIV-1 and *Helicobacter pylori*. *Nat Immunol* 10:1081–1088
 49. Reiling N, Ehlers S, Holscher C (2008) MyDths and un-TOLled truths: sensor, instructive and effector immunity to tuberculosis. *Immunol Lett* 116:15–23
 50. Motshwene PG, Moncrieffe MC, Grossmann JG, Kao C, Ayaluru M, Sandercock AM, Robinson CV, Latz E, Gay NJ (2009) An oligomeric signaling platform formed by the Toll-like receptor signal transducers MyD88 and IRAK-4. *J Biol Chem* 284:25404–25411
 51. Gay NJ, Gangloff M, O'Neill LA (2011) What the Myddosome structure tells us about the initiation of innate immunity. *Trends Immunol* 32:104–109
 52. Bonham KS, Kagan JC (2014) Endosomes as platforms for NOD-like receptor signaling. *Cell Host Microbe* 15:523–525
 53. Bruchard M, Rebe C, Derangere V, Togbe D, Ryffel B, Boidot R, Humblin E, Hamman A, Chalmin F, Berger H, Chevriaux A, Limagne E, Apetoh L, Vegran F, Ghiringhelli F (2015) The receptor NLRP3 is a transcriptional regulator of TH2 differentiation. *Nat Immunol* 16:859–870
 54. Pandey RK, Sodhi A, Biswas SK, Dahiya Y, Dhillon MK (2012) *Mycobacterium indicus pranii* mediates macrophage activation through TLR2 and NOD2 in a MyD88 dependent manner. *Vaccine* 30:5748–5754
 55. Ferwerda G, Girardin SE, Kullberg BJ, Le Bourhis L, de Jong DJ, Langenberg DM, van Crevel R, Adema GJ, Ottenhoff TH, Van der Meer JW, Netea MG (2005) NOD2 and toll-like receptors are nonredundant recognition systems of *Mycobacterium tuberculosis*. *PLoS Pathog* 1:279–285
 56. Master SS, Rampini SK, Davis AS, Keller C, Ehlers S, Springer B, Timmins GS, Sander P, Deretic V (2008) *Mycobacterium tuberculosis* prevents inflammasome activation. *Cell Host Microbe* 3:224–232
 57. Zhou Y, Zhao D, Yue R, Khan SH, Shah SZ, Yin X, Yang L, Zhang Z, Zhou X (2015) Inflammasomes-dependent regulation of IL-1 β secretion induced by the virulent *Mycobacterium bovis* Beijing strain in THP-1 macrophages. *Antonie Van Leeuwenhoek* 108:163–171
 58. Dorhoi A, Nouailles G, Jorg S, Hagens K, Heinemann E, Pradl L, Oberbeck-Muller D, Duque-Correa MA, Reece ST, Ruland J, Brosch R, Tschopp J, Gross O, Kaufmann SH (2012) Activation of the NLRP3 inflammasome by *Mycobacterium tuberculosis* is uncoupled from susceptibility to active tuberculosis. *Eur J Immunol* 42:374–384
 59. Walter K, Holscher C, Tschopp J, Ehlers S (2010) NALP3 is not necessary for early protection against experimental tuberculosis. *Immunobiology* 215:804–811
 60. Sokolovska A, Becker CE, Ip WK, Rathinam VA, Brudner M, Paquette N, Tanne A, Vanaja SK, Moore KJ, Fitzgerald KA, Lacy-Hulbert A, Stuart LM (2013) Activation of caspase-1 by the NLRP3 inflammasome regulates the NADPH oxidase NOX2 to control phagosome function. *Nat Immunol* 14:543–553
 61. Capparelli R, De Chiara F, Di Matteo A, Medaglia C, Iannelli D (2013) The MyD88 rs6853 and TIRAP rs8177374 polymorphic sites are associated with resistance to human pulmonary tuberculosis. *Genes Immun* 14:504–511
 62. Basu J, Shin DM, Jo EK (2012) *Mycobacterial* signaling through toll-like receptors. *Front Cell Infect Microbiol* 2:145
 63. Pathak SK, Basu S, Basu KK, Banerjee A, Pathak S, Bhattacharyya A, Kaisho T, Kundu M, Basu J (2007) Direct extracellular interaction between the early secreted antigen ESAT-6 of *Mycobacterium tuberculosis* and TLR2 inhibits TLR signaling in macrophages. *Nat Immunol* 8:610–618
 64. Kim K, Sohn H, Kim JS, Choi HG, Byun EH, Lee KI, Shin SJ, Song CH, Park JK, Kim HJ (2012) *Mycobacterium tuberculosis* Rv0652 stimulates production of tumour necrosis factor and monocytes chemoattractant protein-1 in macrophages through the Toll-like receptor 4 pathway. *Immunology* 136:231–240
 65. Shi S, Nathan C, Schnappinger D, Drenkow J, Fortes M, Block E, Ding A, Gingeras TR, Schoolnik G, Akira S, Takeda K, Ehrt S (2003) MyD88 primes macrophages for full-scale activation by interferon-gamma yet mediates few responses to *Mycobacterium tuberculosis*. *J Exp Med* 198:987–997
 66. Liu G, Yang H (2013) Modulation of macrophage activation and programming in immunity. *J Cell Physiol* 228:502–512
 67. Fortune SM, Solache A, Jaeger A, Hill PJ, Belisle JT, Bloom BR, Rubin EJ, Ernst JD (2004) *Mycobacterium tuberculosis* inhibits macrophage responses to IFN-gamma through myeloid differentiation factor 88-dependent and -independent mechanisms. *J Immunol* 172:6272–6280
 68. Sugawara I, Yamada H, Mizuno S, Takeda K, Akira S (2003) *Mycobacterial* infection in MyD88-deficient mice. *Microbiol Immunol* 47:841–847
 69. Andersson H, Andersson B, Eklund D, Ngoh E, Persson A, Svensson K, Lerm M, Blomgran R, Stendahl O (2014) Apoptotic neutrophils augment the inflammatory response to *Mycobacterium tuberculosis* infection in human macrophages. *PLoS One* 9:e101514
 70. Fremont CM, Togbe D, Doz E, Rose S, Vasseur V, Maillet I, Jacobs M, Ryffel B, Quesniaux VF (2007) IL-1

- receptor-mediated signal is an essential component of MyD88-dependent innate response to *Mycobacterium tuberculosis* infection. *J Immunol* 179:1178–1189
71. Mayer-Barber KD, Andrade BB, Oland SD, Amaral EP, Barber DL, Gonzales J, Derrick SC, Shi R, Kumar NP, Wei W, Yuan X, Zhang G, Cai Y, Babu S, Catalfamo M, Salazar AM, Via LE, Barry CE 3rd, Sher A (2014) Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. *Nature* 511:99–103
 72. Friedland JS (2014) Targeting the inflammatory response in tuberculosis. *N Engl J Med* 371:1354–1356
 73. Behar SM, Sasseti CM (2014) Immunology: Fixing the odds against tuberculosis. *Nature* 511:39–40
 74. Briken V, Ahlbrand SE, Shah S (2013) *Mycobacterium tuberculosis* and the host cell inflammasome: a complex relationship. *Front Cell Infect Microbiol* 3:62
 75. Dallenga T, Schaible UE (2016) Neutrophils in tuberculosis—first line of defence or booster of disease and targets for host-directed therapy? *Pathog Dis* 74:ftw012
 76. Marzo E, Vilaplana C, Tapia G, Diaz J, Garcia V, Cardona PJ: Damaging role of neutrophilic infiltration in a mouse model of progressive tuberculosis. *Tuberculosis (Edinb)* 2014;94:55–64.
 77. Ruland J (2011) Return to homeostasis: downregulation of NF-kappaB responses. *Nat Immunol* 12:709–714
 78. Burns K, Janssens S, Brissoni B, Olivos N, Beyaert R, Tschopp J (2003) Inhibition of interleukin 1 receptor/Toll-like receptor signaling through the alternatively spliced, short form of MyD88 is due to its failure to recruit IRAK-4. *J Exp Med* 197:263–268
 79. Carpenter S, Aiello D, Atianand MK, Ricci EP, Gandhi P, Hall LL, Byron M, Monks B, Henry-Bezy M, Lawrence JB, O'Neill LA, Moore MJ, Caffrey DR, Fitzgerald KA (2013) A long non-coding RNA mediates both activation and repression of immune response genes. *Science* 341:789–792
 80. Janssens S, Burns K, Vercammen E, Tschopp J, Beyaert R (2003) MyD88S, a splice variant of MyD88, differentially modulates NF-kappaB- and AP-1-dependent gene expression. *FEBS Lett* 548:103–107
 81. Maskrey BH, Megson IL, Whitfield PD, Rossi AG (2011) Mechanisms of resolution of inflammation: a focus on cardiovascular disease. *Arterioscler Thromb Vasc Biol* 31:1001–1006
 82. Araki A, Kanai T, Ishikura T, Makita S, Uraushihara K, Iiyama R, Totsuka T, Takeda K, Akira S, Watanabe M (2005) MyD88-deficient mice develop severe intestinal inflammation in dextran sodium sulfate colitis. *J Gastroenterol* 40:16–23
 83. Fukata M, Michelsen KS, Eri R, Thomas LS, Hu B, Lukasek K, Nast CC, Lechago J, Xu R, Naiki Y, Soliman A, Arditi M, Abreu MT (2005) Toll-like receptor-4 is required for intestinal response to epithelial injury and limiting bacterial translocation in a murine model of acute colitis. *Am J Physiol Gastrointest Liver Physiol* 288:G1055–G1065
 84. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R (2004) Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 118:229–241
 85. Liu N, Montgomery RR, Barthold SW, Bockenstedt LK (2004) Myeloid differentiation antigen 88 deficiency impairs pathogen clearance but does not alter inflammation in *Borrelia burgdorferi*-infected mice. *Infect Immun* 72:3195–3203
 86. Behera AK, Hildebrand E, Bronson RT, Perides G, Uematsu S, Akira S, Hu LT (2006) MyD88 deficiency results in tissue-specific changes in cytokine induction and inflammation in interleukin-18-independent mice infected with *Borrelia burgdorferi*. *Infect Immun* 74:1462–1470
 87. Bolz DD, Weis JJ (2004) Molecular mimicry to *Borrelia burgdorferi*: pathway to autoimmunity? *Autoimmunity* 37:387–392
 88. Su SB, Silver PB, Grajewski RS, Agarwal RK, Tang J, Chan CC, Caspi RR (2005) Essential role of the MyD88 pathway, but non-essential roles of TLRs 2, 4, and 9, in the adjuvant effect promoting Th1-mediated autoimmunity. *J Immunol* 175:6303–6310
 89. Xu G, Zhang Z, Xing Y, Wei J, Ge Z, Liu X, Zhang Y, Huang X (2014) MicroRNA-149 negatively regulates TLR-triggered inflammatory response in macrophages by targeting MyD88. *J Cell Biochem* 115:919–927
 90. Ma C, Li Y, Zeng J, Wu X, Liu X, Wang Y (2014) *Mycobacterium bovis* BCG triggered MyD88 induces miR-124 feedback negatively regulates immune response in alveolar epithelial cells. *PLoS One* 9:e92419
 91. Rajaram MV, Ni B, Dodd CE, Schlesinger LS (2014) Macrophage immunoregulatory pathways in tuberculosis. *Semin Immunol* 26:471–485
 92. Roy A, Eisenhut M, Harris RJ, Rodrigues LC, Sridhar S, Habermann S, Snell L, Mangtani P, Adetifa I, Lalvani A, Abubakar I (2014) Effect of BCG vaccination against *Mycobacterium tuberculosis* infection in children: systematic review and meta-analysis. *BMJ* 349:g4643
 93. Sweeney KA, Dao DN, Goldberg MF, Hsu T, Venkataswamy MM, Henao-Tamayo M, Ordway D, Sellers RS, Jain P, Chen B, Chen M, Kim J, Lukose R, Chan J, Orme IM, Porcelli SA, Jacobs WR Jr (2011) A recombinant *Mycobacterium smegmatis* induces potent bactericidal immunity against *Mycobacterium tuberculosis*. *Nat Med* 17:1261–1268