

# Nuclear Factor $\kappa$ B Activation Pathways During *Mycobacterium tuberculosis* Infection

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**ABSTRACT:** The interactions between pathogens and host cells and the way by which the immune response is modulated during this process ultimately dictate the fate of infection. Host phagocytes exposed to *Mycobacterium tuberculosis* (*M. tuberculosis*) sense microbial-associated molecular patterns and activate a series of signaling pathways. In this setting, activation of the nuclear factor  $\kappa$ B (NF- $\kappa$ B) initiates transcription of several key genes involved in orchestration of antimycobacterial effector functions. This review describes these major pathways that govern the outcome of host phagocytes infected with *M. tuberculosis*. Furthermore, we highlight evidence of how *M. tuberculosis* modulates activation of NF- $\kappa$ B pathways to evade the host antimycobacterial defense.

**KEYWORDS:** Mycobacteria, immune sensors, NF- $\kappa$ B pathways, host defense

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## Introduction

Ineffective activation of immune responses, commonly observed in patients with active tuberculosis (TB), compromises mycobacterial control, leading to disease progression and increased morbidity and mortality. Despite tremendous investments fostering advances in prophylactic and therapeutic strategies from both governmental and private sectors, TB continues to be the leading cause of death due to a single infection agent.<sup>1</sup> Understanding the mechanisms by which *Mycobacterium tuberculosis* (*M. tuberculosis*) drives immune activation and evades antimycobacterial effector functions is of utmost importance to guide improvement of the TB control.

One of the most important molecular discoveries, which caused direct impact on the understanding of the cellular responses to *M. tuberculosis* and to many other stimuli, was the identification of the members of the nuclear factor  $\kappa$ B (NF- $\kappa$ B) protein family. Nuclear factor  $\kappa$ B regulates a wide range of cellular processes by orchestrating transcription of genes, which are required for many key activities, such as cell growth and development, as well as for production of inflammatory and immune mediators. A large variety of signals can trigger activation of the NF- $\kappa$ B complex, including oxidative injuries, exposure to microbial-associated molecular patterns, hormones, and cytokines.<sup>2</sup> Thus, NF- $\kappa$ B complex could be considered a common molecular player that overlaps several pathways, leading to distinct cellular outcomes on stimulation.

In resting cells, NF- $\kappa$ B is found inhibited by binding to I $\kappa$ B (an inhibitory protein). In this context, the complex NF- $\kappa$ B/I $\kappa$ B remains located in the cytosolic compartment, and there is no

binding to nuclear DNA. On extracellular stimuli with either direct identification of *M. tuberculosis* molecular patterns or of cytokines produced by *M. tuberculosis*-infected macrophages, the activation of NF- $\kappa$ B is initiated. Several receptors anchored to plasma membrane, such as Toll-like receptors (TLRs), cytokine receptors, and many others, will recognize their ligands, and then the signal is transduced into the cell through a series of adaptor signaling proteins, which are located in the cytoplasm. This process results in activation of a synchronized signaling cascade leading to proteasome-mediated degradation of I $\kappa$ B and consequently translocation of NF- $\kappa$ B to cellular nucleus where it activates gene transcription. Nuclear factor  $\kappa$ B signaling is critical for cell response against *M. tuberculosis* and its inhibition or dysfunction is detrimental for the host defense. In this review, we summarize the current knowledge concerning the requirements for NF- $\kappa$ B signaling and its importance for immune response against *M. tuberculosis* infection.

## NF- $\kappa$ B Signaling Overview

In humans, NF- $\kappa$ B family contains 5 members, p65 (RelA), RelB, c-Rel, p50 (p105 precursor), and p52 (p100 precursor),<sup>2,3</sup> which are encoded by *RELA*, *RELB*, *REL*, *NFKB1*, and *NFKB2* genes, respectively. These molecules share a N-terminal Rel homology domain (RHD), which is required for DNA binding and oligomerization. Another subunit of NF- $\kappa$ B that is observed only in p65, c-Rel, and RelB is called transcription activation domain (TAD) and it is necessary for positive regulation of gene expression. In contrast, p50 and p52 lack TADs,



and for this reason, they are unable to transduce the original transcription signal (Figure 1).

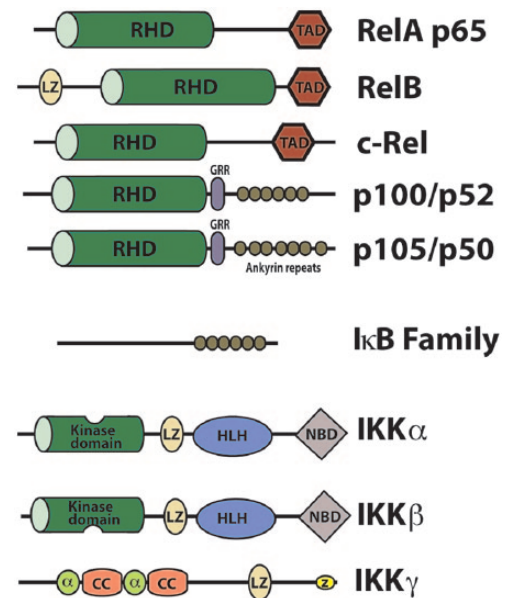
In many cells, NF- $\kappa$ B proteins are found as homodimeric or heterodimeric structures inside cytoplasm and, commonly, are bound to inhibitory proteins named I $\kappa$ Bs. Basically, I $\kappa$ B binds to the RHD region of the NF- $\kappa$ B, compromising its contact to DNA.<sup>4</sup> Three typical I $\kappa$ B proteins, such as I $\kappa$ B $\alpha$  (encoded by *NFKB1A*), I $\kappa$ B $\beta$  (encoded by *NFKB1B*), and I $\kappa$ B $\epsilon$  (encoded by *NFKB1E*), or the precursor proteins, such as p100 (encoded by *NFKB2*) and p105 (encoded by *NFKB1*), are found bound to NF- $\kappa$ B in its inactive stage. Nuclear factor  $\kappa$ B can be activated by several pathways during which it converts to a 700- to 900-kDa complex containing a serin-specific I $\kappa$ B kinase (IKK), designated IKK complex. This complex consists of 2 catalytic subunits, IKK $\alpha$  and IKK $\beta$ , forming homodimer or heterodimer, which are stoichiometrically bound to a regulatory subunit named NEMO (NF- $\kappa$ B essential modifier) or IKK $\gamma$ <sup>5</sup> (Figure 1). Both IKK $\alpha$  and IKK $\beta$  present a site for binding for NEMO named NEMO-binding domain (NBD) (Figure 1). In this scenario, NEMO interacts with IKK dimer conformation and stabilizes the whole structure. Nuclear factor  $\kappa$ B essential modifier is known to be a regulatory subunit of the IKK complex and displays an important domain required for binding to IKK $\beta$  named kinase-binding domain. Interestingly, macrophages from a patient presenting with a novel hypomorphic NEMO mutation were recently shown to exhibit increased susceptibility to *M. tuberculosis* infection.<sup>6</sup> Persons with immunodeficiency related to NEMO dysfunction frequently develop bacille Calmette-Guérin (BCG) infection and failure of I $\kappa$ B in lymphocytes.<sup>6</sup>

The IKK complex is responsible for the phosphorylation of 2 serine residues (Ser32/36) of I $\kappa$ B,<sup>7-9</sup> which drives I $\kappa$ B for degradation. The degradation of I $\kappa$ B releases NF- $\kappa$ B proteins from their inhibitory complex, which facilitates the translocation of activated NF- $\kappa$ B to the nucleus.<sup>3,10</sup> Nuclear factor  $\kappa$ B becomes activated under a wide variety of cellular stimuli by 2 distinct pathways named canonical and noncanonical NF- $\kappa$ B pathways. Briefly, canonical pathway requires the phosphorylation of I $\kappa$ B $\alpha$  dependent on IKK $\beta$  activity, whereas noncanonical NF- $\kappa$ B pathway is regulated by an IKK $\alpha$  homodimer.<sup>3</sup> Both NF- $\kappa$ B pathways and their implications during *M. tuberculosis* infection are dissected below.

### Canonical NF- $\kappa$ B pathway

Pathogen-associated molecular patterns and danger-associated molecular patterns (DAMPs) represent major triggers of the NF- $\kappa$ B canonical pathway activation. Two classical cascades triggered by the tumor necrosis factor receptor (TNFR) superfamily and TLR/interleukin (IL)-1R superfamily are used here to illustrate this process.

*Signaling via the TNFR superfamily.* Tumor necrosis factor receptor superfamily is composed of at least 19 ligands and 29



**Figure 1.** Molecular members of the nuclear factor  $\kappa$ B (NF- $\kappa$ B) pathway. Schematic representation of domain structures of NF- $\kappa$ B family members with indication of their functional domains and their direct modulators I $\kappa$ B and I $\kappa$ B kinase (IKK). All members of NF- $\kappa$ B family share a common domain named N-terminal Rel homology domain (RHD) which is critical for binding to DNA site, protein dimerization, and interaction with I $\kappa$ B. Rel subfamily—RelA, RelB, and c-Rel—displays a transcriptional activation domain (TAD) in their C-terminal portion, and the subunit RelB has an additional leucine zipper (LZ) in its N-terminal site. NF- $\kappa$ B members, p50 and p52, resulted from the processing of p105 and p100 in their C-terminal portion, respectively. I $\kappa$ B proteins contain ankyrin-repeat motifs (ANK) in their C-terminal region (also found on p100 and p105) which interact with the RHD of NF- $\kappa$ B proteins, preventing their nuclear translocation. Between the RHD and ANK lies a glycine-rich region (GRR). Upon activation, GRR provide signal for cleavage of p105 and possibly p100 by another protease, resulting in the release of p50 and p52 and the C-terminus which is ubiquitinated and degraded by the proteasome. IKK complex is primarily composed of the 2 catalytic subunits IKK $\alpha$  and IKK $\beta$  and the scaffolding protein IKK $\gamma$  (or NF- $\kappa$ B essential modifier [NEMO]). IKK $\alpha$  and IKK $\beta$  are structurally related, displaying an LZ domain, a helix-loop-helix region (HLH), and NEMO-binding domain (NBD). IKK $\gamma$  has an alpha helical region along with 2 coiled-coil (CC) regions and a zinc finger (ZF) domain at the C-terminal portion.

receptors and displays variability in terms of expression and physiological action in different tissues.<sup>11</sup> Tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) is a widely studied member of this cytokine family and plays multiple roles in both innate and adaptive immune responses. It is well established that TNF signaling regulates the expression of antiapoptotic genes, such as *cIAP1/2* and *Bcl-XL*, through activation of NF- $\kappa$ B,<sup>12</sup> evoking pro-survival signals. In contrast, under inhibition of NF- $\kappa$ B signaling, TNFR activation triggers a cascade that rapidly drives apoptotic cell death through its cytosolic domain TNFR-associated death domain (TRADD). In other settings, TNFR signaling induces necroptosis under inhibition of caspase 8.<sup>13-15</sup> Because TNF signaling has been implicated in the pathogenesis of many diseases, the clinical use of anti-TNF antibodies opened important perspectives for the treatment of inflammatory diseases, such as rheumatoid arthritis.<sup>16-18</sup> However, the administration of anti-TNF

antibodies has been described to promote the reactivation of other diseases, such as TB, facilitating the dissemination of the bacilli and consequently the disease progression. For these reasons, understanding TNF- $\alpha$ -mediated activation of NF- $\kappa$ B continues to be an important focus of research in numerous laboratories.

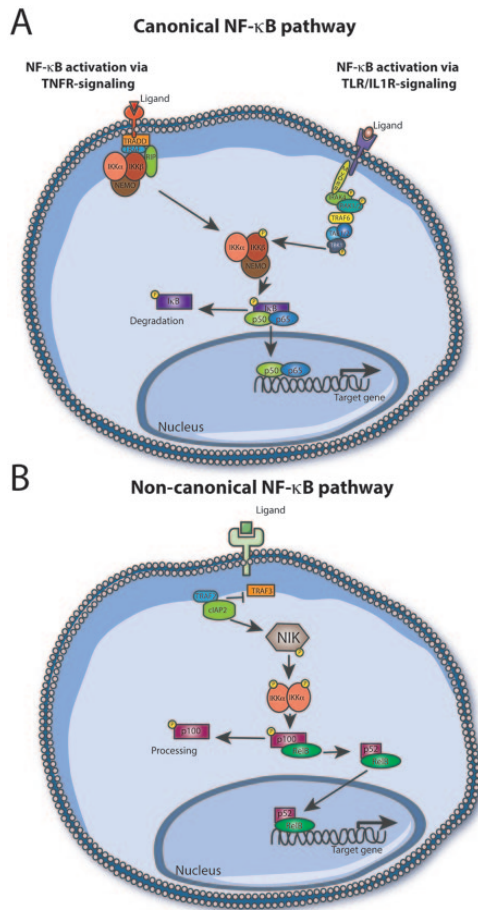
Trimeric TNF- $\alpha$  ligation to TNFR1 is needed for the aggregation of the receptor and association of silencer of death domain (SODD), an endogenous inhibitor of the TNFR activity, allowing the binding of TRADD.<sup>19</sup> Despite its supposed importance for TNF signaling, it has been reported that SODD-deficient mice still present marginal effects on TNF- $\alpha$  signaling to NF- $\kappa$ B canonical pathway,<sup>20,21</sup> suggesting that a redundant signaling cascade may account for activation of this pathway. The TNFR-associated factor (TRAF) family represents an important and critical set of adaptor molecules that are required for the transduction of TNF signal. TRAF2, TRAF3, and TRAF5 have been implicated in TNF signaling. However, these molecules apparently present redundant functions with each other. Indeed, mice deficient in TRAF2 or TRAF5 display intact TNF signaling through NF- $\kappa$ B,<sup>22</sup> whereas TRAF2/5 double knockout cells show substantially reduced TNF-induced IKK activation.<sup>22–24</sup> Through interaction of TRAF with TRADD, TRAF2 recruits the IKK complex via a direct interaction with the leucine zipper region of IKK $\alpha$  or IKK $\beta$ . TRAF2 also interacts with a receptor-interacting protein 1 (RIP1), which can also be independently recruited to TRADD. Receptor-interacting protein 1 kinase is crucial for TNF-induced NF- $\kappa$ B activation,<sup>25</sup> and RIP-deficient cells are susceptible to apoptosis after TNF- $\alpha$  stimulation.<sup>26</sup> Interestingly, RIP works as a scaffold protein, binding directly to NEMO, promoting the recruitment of IKK complex to TNFR independently of TRAF2.<sup>27</sup> Despite this role, the recruitment of IKK complex via TRAF2 independently of RIP2 is not sufficient for IKK activation,<sup>28</sup> suggesting the crucial role of RIP in TNF-induced canonical NF- $\kappa$ B pathway. In both cases, RIP may nucleate the assembly of signaling complex that induces IKK activation via oligomerization of NEMO and then autophosphorylation of IKK.<sup>29</sup> The phosphorylation of IKK $\beta$  is a hallmark of canonical pathway because its activity will guarantee the phosphorylation of I $\kappa$ B and release of p65 to translocate into the nucleus.

*Signaling through the TLR/IL-1R superfamily.* Toll-like receptor signaling has been reported to play a fundamental role in mycobacterial recognition by macrophages and other myeloid cell types.<sup>30,31</sup> Toll-like receptor-driven activation of innate immune cells is crucial for the control of bacterial proliferation and for adequate activation of cells from adaptive immunity. Also, in addition to recognizing pathogens, TLRs can sense danger signals released from dead cells, such as heat shock proteins (HSP) and high-mobility group box 1 (HMGB-1).<sup>32–35</sup> Thus, TLRs display important protagonism in self-nonself decisions, playing a central role in the innate immunity, as well as in the initiation of adaptive immune responses. Distinct

from TLRs, IL-1R recognizes immature/mature IL-1 $\alpha$  and IL-1 $\beta$ , and its signaling was reported to be requested for host protection in several infections, including mycobacterial infection.<sup>36–38</sup> Interestingly, both TLR and IL-1R pathways activate NF- $\kappa$ B through canonical pathway. Despite exhibiting distinct extracellular portion, TLRs and IL-1R bear strong homology to their intracellular portions, sharing the Toll/IL-1R (TIR) domain,<sup>39,40</sup> which mediates interaction with downstream signaling adaptors required for the activation of transcription factors, such as NF- $\kappa$ B, AP-1, and IRF3.<sup>41</sup> The adaptor protein shared by TLRs/IL-1R and which is responsible for that signaling transduction is called MyD88. All TLRs require engagement of MyD88 to initiate downstream cascade, except TLR3 which engages only TIR domain-containing adaptor-inducing interferon  $\beta$  (TRIF) as adaptor protein. MyD88 interacts directly with TIR domains on TLRs or IL-1R cytoplasmic tails, and it is fundamental for normal NF- $\kappa$ B induction in their response to its ligands.<sup>39,41–43</sup> Once bound to the TIR domain, MyD88 connects with members of the IL-1R-associated kinase (IRAK) family,<sup>41,44,45</sup> including IRAK1, IRAK2, IRAK4, and IRAK-M. The large oligomeric complex that resulted from the assembling of MyD88 and IRAKs is named Myddosome.<sup>46</sup> Studies in genetically deficient mice reported that IRAK1, IRAK2, and IRAK4 serve as activators, whereas IRAK-M acts as a negative regulator of the NF- $\kappa$ B canonical pathway.<sup>47–51</sup> Monarch-1 (also known as NLRP12) is also known to be an important negative regulator of the NF- $\kappa$ B pathway by binding to IRAK1/2, and not MyD88, which results in the blockage of IRAK1/2 hyperphosphorylation, avoiding NF- $\kappa$ B activation during *M. tuberculosis* infection.<sup>52,53</sup> Instead, phosphorylated IRAK4 induces IRAK1/2 phosphorylation.<sup>54,55</sup> Once phosphorylated, IRAK1/2 promotes engagement of TNFR-associated factor 6 (TRAF6), an E3 ubiquitin ligase, to the TLR/IL-1R-signaling complex on the cytoplasmic side of cell membrane.<sup>56–59</sup> This activation step is critical for MyD88-dependent activation of NF- $\kappa$ B<sup>53,56,60,61</sup> because TRAF6-deficient cells show impaired NF- $\kappa$ B activation under stimulation of IL-1R and TLR4, directly affecting cytokine production.<sup>62,63</sup> On activation, TRAF6 induces docking and activation of the TAB1, TAB2, and TAB3 adaptors, leading to the recruitment of serine-threonine kinase TAK1 into the complex named TAK complex.<sup>64</sup> TAK1 requires TAB1 for its kinase activity.<sup>65,66</sup> TAB2 and TAB3 recognize Lys-63-polyubiquitin chains that are found anchored on TRAF6, bringing the kinase domain of TAK1 near each other.<sup>67</sup> Thus, TAK1 promotes its autophosphorylation and activation, which in turn can phosphorylate and activate IKK $\beta$ , culminating on I $\kappa$ B proteasomal degradation and NF- $\kappa$ B translocation into the nucleus.<sup>68,69</sup> TNFR-associated factor 6 has also been reported to induce RIP1 kinase recruitment into the complex triggered by TLR3/TRIF, allowing the activation of NF- $\kappa$ B by RIP1 kinase<sup>70</sup> (Figure 2A).

### *Noncanonical NF- $\kappa$ B pathway*

Activation of both canonical and noncanonical NF- $\kappa$ B pathways involves ubiquitination of its inhibitor I $\kappa$ B by the IKK complex or semiproteolysis of p100 by IKK $\alpha$  homodimer.<sup>71,72</sup>



**Figure 2.** Activating nuclear factor  $\kappa$ B (NF- $\kappa$ B) pathways. Schematic illustration of (A) canonical and (B) noncanonical NF- $\kappa$ B pathways. (A) Tumor necrosis factor (TNF)-dependent (on left) and Toll-like receptor (TLR)/interleukin (IL)-1R-dependent (on right) NF- $\kappa$ B signaling pathways are represented. Once triggered, tumor necrosis factor receptor (TNFR) (left) recruits TNFR-associated factor (TRAF) family that represents a critical adaptor molecule required for the transduction of TNF signal. Already anchored to TNFR-associated death domain (TRADD), TRAF recruits IKK complex via a direct interaction with the leucine zipper region of IKK $\alpha$  or IKK $\beta$ . TRAF also interacts with receptor-interacting protein 1 (RIP1) which can be independently recruited to TRADD. In the last case, RIP works as a scaffold protein, binding directly to NEMO, promoting the recruitment of IKK complex to TNFR. TLR/IL-1R (on right) when activated recruits MyD88 by binding to their TIR domain. Once bound to TIR domain, MyD88 connects with IRAK members, resulting in a large oligomeric complex named Myddosome. Phosphorylated IRAK4 induces IRAK1/2 phosphorylation, which in turn promotes engagement of TRAF6. On activation, TRAF6 induces docking and activation of the TAB1, TAB2, and TAB3 adaptors, leading to the recruitment of TAK1. In turn, TAK1 promotes its autophosphorylation and then phosphorylates IKK $\beta$ . Activated IKK complex phosphorylates I $\kappa$ B, leading to its degradation by the proteasome. On I $\kappa$ B degradation, NF- $\kappa$ B subunits will be released and then allowed to translocate into the nucleus. (B) Noncanonical NF- $\kappa$ B signaling has a central signaling component named nuclear factor  $\kappa$ B-inducing kinase (NIK) which acts together with IKK $\alpha$  to induce phosphorylation and processing of p100. For NIK activation, TRAF3, a negative regulation of NIK expression, is downregulated by cIAP2 and TRAF2, facilitating NIK accumulation in the cytosol and its transphosphorylation. As it is activated, NIK phosphorylates IKK $\alpha$ , which in turn promotes p100 processing, allowing RelB/p52 translocation into the nucleus.

Canonical NF- $\kappa$ B pathway has been implicated in mediating proinflammatory responses in innate immune cells, whereas the noncanonical pathway (RelB/p50 or RelB/p52 proteins) is associated classically with the regulation of secondary lymphoid organ development and acquired immunity.<sup>73</sup> The major reason for this attribution of noncanonical pathway is because this pathway is strongly induced under B cell-activating factor receptor (BAFF-R) and lymphotoxin beta receptor (LT- $\beta$ R) stimulation.<sup>74</sup> However, there is evidence indicating that noncanonical NF- $\kappa$ B pathway is required for the regulation of macrophage and dendritic cell (DC) activation. Gasparini and colleagues have recently demonstrated that RelB/p50 regulates TNF- $\alpha$  production in both macrophages and DC on lipopolysaccharide (LPS) stimulation.<sup>75</sup> Lipopolysaccharide is widely known to trigger TLR4 and induce macrophage and DC activation through canonical pathway. However, during later time points after LPS stimulation, RelB involvement seems to play a role in induction of CCL19,<sup>76</sup> a homeostatic chemokine relevant to DC interaction with T cells, as well as in inhibiting TNF- $\alpha$  generation but not affecting IL-6 production in macrophages.<sup>75</sup> In addition, noncanonical NF- $\kappa$ B pathway is suggested to be crucial to maintain CXCL12 autocrine loop in macrophages in response to HMGB1, affecting cell migration.<sup>77</sup>

Some specific subsets of TNFR superfamily members are described to induce noncanonical NF- $\kappa$ B pathway, such as LT- $\beta$ R, CD40 BAFF-R, receptor activator of nuclear factor  $\kappa$ B (RANK), TNFR2, and CD27.<sup>78–82</sup> Nuclear factor  $\kappa$ B-inducing kinase (NIK) is the central signaling component of the noncanonical NF- $\kappa$ B pathway and acts together with IKK $\alpha$  to induce phosphorylation-dependent ubiquitination and processing of p100.<sup>83,84</sup> Under unstimulated conditions, the expression of NIK is maintained at low levels, but after stimulation through some receptors from the TNFR superfamily, NIK expression is upregulated.<sup>85</sup> Downregulation of NIK under resting condition occurs because TRAF3 can interact directly with NIK, leading to its degradation. Another manner for regulation of noncanonical NF- $\kappa$ B pathway is mediated by Monarch-1/NLRP12 through association with NIK, inducing its proteasome-mediated degradation.<sup>86,87</sup> For NIK activation, TRAF3 needs to be downregulated, which is facilitated by cIAP2 and TRAF2 under noncanonical NF- $\kappa$ B inducers.<sup>88</sup> TRAF2 activates cIAP2, which in turn targets TRAF3 for K48 ubiquitination and degradation, leading to NIK accumulation and its transphosphorylation.<sup>82,84</sup> Once phosphorylated, NIK activates IKK $\alpha$  homodimer, which promotes p100 processing, culminating in nuclear translocation of RelB/p52 (Figure 2B).

### Modulation of Canonical and Noncanonical NF- $\kappa$ B Pathways During Mycobacterial Infection

Nuclear factor  $\kappa$ B activation plays a key role in the immune responses against *M. tuberculosis*. Indeed, mice with genetic

disruption of NF- $\kappa$ B subunit p50 are dramatically more susceptible to *M. tuberculosis* infection compared with wild-type (WT) mice.<sup>89</sup> After reaching the lungs, the *M. tuberculosis* bacilli are recognized by pattern recognition receptors (PRRs) expressed on the surface of alveolar macrophages, which represent the main cell population in the front line of host defense in pulmonary infection.<sup>30</sup> Mutations in genes transcribing PRRs or molecules required for their downstream signaling lead to increased host susceptibility to TB in humans and mice.<sup>30,31</sup> Among TLRs, TLR2 recognizes mycobacterial glycolipids, such as lipomanan, lipoarabinomannan, 19-kDa glycoprotein, phosphatidylinositol mannoside, triacylated and diacylated lipoprotein, as well as early secreted antigenic target of 6-kDa (ESAT-6) protein.<sup>90,91</sup> Work by Richardson and colleagues has demonstrated that *M. tuberculosis* induces rapid TLR2-dependent NF- $\kappa$ B activation, which is evidenced by I $\kappa$ B $\alpha$  degradation in infected WT macrophages, but not in those deficient in TLR2.<sup>92</sup> TLR2 also recognizes DAMPs, such as HMGB1 released from necrotic cells, representing a mechanism that regulates apoptosis during *M. tuberculosis* infection.<sup>33,93,94</sup> TLR4 recognizes the mycobacterial HSP, mitochondrial 50S ribosomal protein, and 38-kDa glycoprotein.<sup>95–98</sup> TIR domain-containing adaptor-inducing interferon  $\beta$ /MyD88 signaling is responsible for NF- $\kappa$ B activation in response to *M. tuberculosis* Hsp70-mediated activation of DCs.<sup>98</sup> TLR9 has been described to recognize mycobacterial DNA in the endosome and phagosomes, cooperating with TLR2 in mediating optimal host resistance to *M. tuberculosis* infection.<sup>99</sup> Mice deficient in TLR2, TLR4 or TLR9, TLR2/9 double deficient or TLR2/4/9 triple deficient, are reported to be more susceptible to *M. tuberculosis* infection than WT animals.<sup>99–102</sup> Importantly, all these TLRs require the engagement of MyD88, an important adaptor protein for NF- $\kappa$ B signaling. Mice deficient in MyD88 are highly susceptible to *M. tuberculosis* infection,<sup>36,100,103,104</sup> confirming the essential role of NF- $\kappa$ B pathway for host protection in TB. In addition, the signaling through the TLR2/MyD88 pathway has been reported to aid confining avirulent mycobacteria inside phagolysosomes during macrophage infection. Such roles have not been observed for virulent mycobacterial strains, suggesting that virulent *M. tuberculosis* may subvert TLR2/MyD88 pathway and thus favor their escape from phagosomes into cytosol.<sup>105</sup> Interestingly, MyD88 does not only play significant role in TLR signaling, but it is also required for signal transduction triggered by IL-1R. By infecting IL-1R-deficient mice, Fremont and colleagues<sup>106</sup> demonstrated that IL-1R-mediated signal is a crucial component of MyD88-dependent innate response to *M. tuberculosis* infection. These findings were further confirmed by Mayer-Barber et al.<sup>36,38</sup>

In addition to its critical roles in innate immune responses, the MyD88 pathway is also important for effectiveness of adaptive immunity. In agreement with this idea, MyD88 has been reported to contribute to Th1 polarization of activated T cells once it is required for IL-12 induction.<sup>92</sup> Virulent

*M. tuberculosis* drives anti-inflammatory responses in infected macrophages and inhibits Th1 polarization of T cells through induction of extracellular signal-regulated kinase (ERK) phosphorylation, apart from inducing NF- $\kappa$ B activation. Pharmacologic inhibition of ERK, as well as genetic deficiency of *Tp12* (required for ERK activation), has been shown to result in lower IL-10 production and enhanced IL-12 secretion by *M. tuberculosis*-infected macrophages, which skews adaptive immune response to a Th1 profile.<sup>92</sup> However, the recombinant proteins TB9.8 from *Mycobacterium bovis* and PPE57 from *M. tuberculosis* have been described to induce high levels of IL-6 and IL-12p40 in macrophage cultures through activation of p38, ERK, and NF- $\kappa$ B signaling pathways.<sup>107,108</sup> Overexpression of PPE57 in BCG strain improves protective host response against *M. tuberculosis* compared with BCG vaccine strain, suggesting that TLR2/MyD88/NF- $\kappa$ B pathway activation through PPE57 may positively regulate innate and adaptive immune responses.<sup>108</sup> Moreover, a proline-glutamate (PE) named PE27 (Rv2769), the major PE member of PE family, was recently described to induce DC maturation through a mechanism dependent on NF- $\kappa$ B and mitogen-activated protein kinase (MAPK) activation, resulting in increased expression of CD80, CD86, major histocompatibility complex (MHC) class I and MHC class II, as well as of proinflammatory cytokines, such as TNF- $\alpha$ , IL-6, and IL-12p70. This phenotype favors induction of IFN- $\gamma$ -producing memory T-cell responses in *M. tuberculosis*-infected mice, suggesting that PE27 may be an important molecule from *M. tuberculosis* to elicit Th1 polarization in the host.<sup>109</sup> Thus, bacterial virulence factors modulate macrophage responses by tipping the balance between activation of NF- $\kappa$ B and ERK pathways, which can directly influence the efficacy of the antimycobacterial immune responses.

*Mycobacterium tuberculosis* is also recognized by binding to dectin-1, which enhances IL-12p40 production by splenic DCs through NF- $\kappa$ B activation.<sup>110</sup> However, single deficiency of dectin-1 was not required for host resistance to *M. tuberculosis* in a murine model of TB.<sup>111</sup> A possible explanation for these discrepancies is that dectin-1 plays relevant role in the cytokine production when it is associated with TLR2.<sup>112–115</sup> Once activated, dectin-1 induces the recruitment of salt-inducible kinase (SIK) via a single Src homology 2 domain (SH2). In turn, phosphorylated SIK induces the recruitment of the CARD9/Bcl-10/Malt1 complex, which phosphorylates I $\kappa$ B $\alpha$ , facilitating nuclear translocation of NF- $\kappa$ B via its canonical pathway while also promoting NIK phosphorylation, required for the activation of the noncanonical NF- $\kappa$ B pathway.<sup>114,116</sup> Although dectin-1 is described to induce both canonical and noncanonical NF- $\kappa$ B pathways, the importance of both intracellular pathways elicited by dectin-1 in the context of *M. tuberculosis* infection remains unclear. Another C-type lectin receptor named mincle has been reported to recognize the mycobacterial glycolipid trehalose dimycolate (cord factor). Recently, mincle/CARD9 signaling has been

implicated in the induction of Th17-promoting adjuvant activity of mycobacterial antigens by facilitating the inflammasome activation.<sup>117,118</sup>

The role of noncanonical NF- $\kappa$ B pathway activation triggered by LT- $\beta$ R and CD40L in TB has been reported.<sup>119,120</sup> Ehlers and colleagues have shown that deficiency of LT- $\beta$ R in mice increases susceptibility to *M. tuberculosis* infection, evidenced by higher bacterial burden in the lung, widespread pulmonary necrosis, and increased mortality rates than in WT mice.<sup>119</sup> Moreover, macrophages from a CD40L-deficient patient have been shown to exhibit defective control of mycobacterial growth, which was restored by treatment with exogenous recombinant IFN- $\gamma$ .<sup>120</sup> In this study, IFN- $\gamma$  treatment induced expression of TLR1/2, dectin-1, and DC-SIGN in macrophages, enhancing resistance against *M. tuberculosis* intracellular growth.<sup>120</sup>

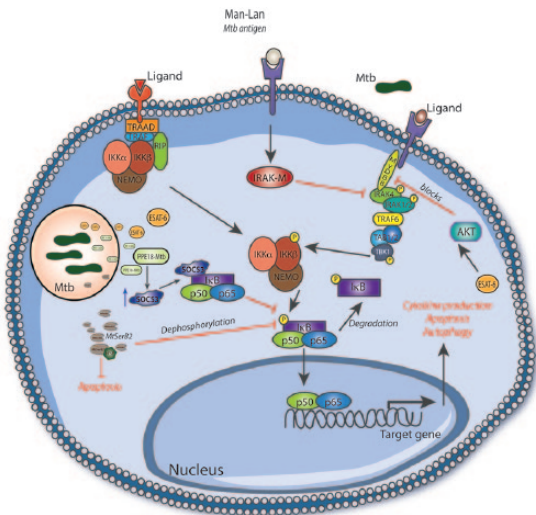
Autophagy induction has been described to limit mycobacterial infection.<sup>121–123</sup> Van der Vaart and colleagues reported that mycobacterial infection induces expression of *DRAM1* (DNA damage-regulated autophagy modulator), an important regulator of autophagy. *DRAM1* expression has been shown to depend on NF- $\kappa$ B activation triggered by MyD88 signaling through TLR/IL-1R. Overexpression of *DRAM1* in infected macrophages induces hyperactivation of autophagy, which has been shown to limit the intracellular mycobacterial growth, whereas depletion of *DRAM1* expression is associated with augmented mycobacterial infection.<sup>124</sup> Moreover, the metabolic switch from basal metabolism to aerobic glycolysis after *M. tuberculosis* infection is thought to depend on TLR2 signaling and mediated in part via activation of AKT-mTOR pathway, another important regulator of autophagy.<sup>125</sup> A recent study reported that *M. tuberculosis* Rv2185c protein exhibits potential to activate NF- $\kappa$ B in macrophages, and this activation has been shown to increase proapoptotic gene expression and drive apoptosis.<sup>126</sup> In addition, PE13 (Rv1195), a member of PE/PPE family protein expressed in mycobacterial surface, has been implicated in modulation of the macrophage activation and apoptosis via a mechanism dependent of p38/ERK/NF- $\kappa$ B axis.<sup>127</sup>

Interestingly, the activation of TLR2-dependent canonical NF- $\kappa$ B pathway has been described to induce microRNA-155 (miR-155) expression in macrophages infected with mycobacteria.<sup>128</sup> Following in vivo *M. tuberculosis* infection, miR-155-deficient mice display increased susceptibility to infection, evidenced by higher mortality rates and higher bacterial burdens in the lungs compared with WT mice.<sup>129</sup> Among other biological roles, miR-155 is required for induction of heme oxygenase 1 (HO-1) by reducing protein translation of the transcription factor Bach1, which is a host repressor of Nrf2 (a transcription factor known to induce HO-1 messenger RNA transcription). Heme oxygenase 1 is an important biomarker for active TB infection, and its induction in macrophages is dependent on exposure to live replicating mycobacteria, as well as production of ESAT-6 by *M. tuberculosis*.<sup>130–132</sup> Lung tissue damage in active TB involves the degradation of lung

extracellular matrix induced by matrix metalloproteinases (MMPs).<sup>133–135</sup> We have recently reported that *M. tuberculosis*-induced HO-1 signaling is required for CO generation, which in turn inhibits MMP expression by preventing the activation of c-Jun/AP1.<sup>132</sup> Reduction in MMP activity could potentially be used to reduce extensive lung damage pathology, which hallmarks pulmonary TB. In agreement with this idea, a recent study demonstrated that pharmacologic inhibition of MMP with BB-94 administration in murine model of TB reduced bacterial burden in the lung, increased collagen deposition in the early granuloma, and reduced leukocyte recruitment into the lung.<sup>136</sup> However, BB-94 is also known to inhibit members of the a disintegrin and metalloproteinase (ADAM) family including ADAM-17, a TNF- $\alpha$ -cleaving enzyme that is required for TNF- $\alpha$  release, which makes interpretation of the data on MMP inhibition difficult.<sup>137</sup> However, the inhibition of HO-1 expression in CD4<sup>+</sup> T cells was shown to improve antigen-specific T-cell expansion and effector function of this T-cell subset in different experimental settings.<sup>138,139</sup> However, the requirement of HO-1 modulation for the activation of CD4<sup>+</sup> T cells, as well as NF- $\kappa$ B activation, in this process during mycobacterial infection remains unclear.

Despite the established ability of *M. tuberculosis* to induce NF- $\kappa$ B activation, some *M. tuberculosis* antigens have been shown to inhibit NF- $\kappa$ B signaling as a manner to evade the host defense in some conditions. Thus, the balance in host-pathogen interactions that activate or modulate NF- $\kappa$ B pathways can ultimately determine the outcome of infection. Through suppressing NF- $\kappa$ B activation, *M. tuberculosis* favors production of anti-inflammatory cytokines by epithelial cells, such as IL-22 and IL-10.<sup>140</sup> Shree and colleagues<sup>141</sup> reported that Rv3042c (also known as MtSerB2), a bacterial haloacid dehalogenase phosphatase, interacts with HSP90, HSP70, and HSP27 and inhibits apoptotic pathways in host cells, which in turn has been shown to be required for mycobacterial elimination.<sup>142</sup> Indeed, MtSerB2 dephosphorylates NF- $\kappa$ B and MAPK-p38, which results in cell deactivation and failure of host resistance against *M. tuberculosis*. Pharmacologic inhibition of MtSerB2 with clofazimine, a drug used for the treatment of extensively drug resistant and multidrug resistant TB cases, is thought to successfully restore antimycobacterial cellular response,<sup>141</sup> reinforcing the idea that *M. tuberculosis* directly dampens NF- $\kappa$ B activation to suppress host resistance.

PPE18-*M. tuberculosis* antigen has been reported to inhibit LPS-stimulated macrophage activation by blocking NF- $\kappa$ B activation. Essentially, PPE18 limits NF- $\kappa$ B activation on macrophage cultures by inducing upregulation of suppressor of cytokine signaling 3 (SOCS3). Suppressor of cytokine signaling 3 binds to I $\kappa$ B $\alpha$ , inhibiting its phosphorylation and thus preventing the nuclear translocation of NF- $\kappa$ B in LPS-primed macrophages. Downregulation of SOCS3 expression using RNA interference drives I $\kappa$ B $\alpha$  phosphorylation, as well as nuclear NF- $\kappa$ B translocation in macrophages costimulated by PPE18 and LPS,<sup>143</sup> reinforcing this mechanistic link. Man-LAN from *M. tuberculosis* was also reported to inhibit



**Figure 3.** Nuclear factor  $\kappa$ B (NF- $\kappa$ B) signaling modulation by mycobacteria. Several *M. tuberculosis* antigens can induce NF- $\kappa$ B activation through their recognition by pattern recognition receptors and also by eliciting cytokines receptors, such as tumor necrosis factor receptor and interleukin-1R, culminating in protective cytokine production, apoptosis induction, and autophagy modulation. However, some *M. tuberculosis* antigens can inhibit NF- $\kappa$ B activation as mechanism to evade the host defense. MtSerB2, a bacterial haloacid dehalogenase phosphatase, interacts with HSP90, HSP70, and HSP27 and inhibits apoptotic pathways in host cells, facilitating bacterial survival. Also, MtSerB2 dephosphorylates NF- $\kappa$ B and mitogen-activated protein kinase-p38, which results in cell deactivation. Another *M. tuberculosis* antigen named PPE18 limits NF- $\kappa$ B activation on macrophage cultures by inducing upregulation of suppressor of cytokine signaling 3 (SOCS3), which in turn binds to I $\kappa$ B $\alpha$ , preventing its phosphorylation which maintains NF- $\kappa$ B in the cytosol. *M. tuberculosis* Man-LAN recognition induces IRAK-M expression, an important negative regulator of the classical NF- $\kappa$ B signaling. *M. tuberculosis* early secreted antigenic target of 6-kDa (ESAT-6) protein is an important virulence factor of *M. tuberculosis* and has been described to inhibit NF- $\kappa$ B pathway by targeting kinase Akt activation, which in turn prevents the interaction between MyD88 and IRAK4.

macrophage activation by enhancing IRAK-M expression, an important negative regulator of the classical NF- $\kappa$ B pathway.<sup>144</sup> In this study, IRAK-M genetic knockdown restored IL-12 production by macrophages costimulated with LPS and Man-LAN.<sup>144</sup> Furthermore, *M. tuberculosis* ESAT-6 can inhibit NF- $\kappa$ B pathway by targeting kinase Akt activation, which in turn prevents the interaction between MyD88 and IRAK4.<sup>145</sup> Downregulation of Monarch-1/NLRP12 expression, a negative regulator of canonical and noncanonical NF- $\kappa$ B pathway,<sup>52,86,87</sup> culminates in enhancement of IL-6 and IL-12 production by macrophages in response to virulent *M. tuberculosis* infection,<sup>52,146</sup> revealing that *M. tuberculosis* can induce Monarch-1/NLRP12 expression which in turn modulates NF- $\kappa$ B activation (Figure 3).

### Concluding Remarks

Complex interactions between *M. tuberculosis* and host cells dictate whether NF- $\kappa$ B pathways are activated or suppressed; such molecular determinants are associated with the fate of infected cells and ultimately affect the TB disease outcomes in

vitro and in vivo. Manipulation of NF- $\kappa$ B pathways to subvert microbial evasion mechanisms and enhance antimycobacterial immune responses could serve as potential tool to reduce TB disease burden. However, interfering in these pathways is difficult and poses huge risk for pleiotropic effects, given that NF- $\kappa$ B is involved in several other metabolic pathways which are essential for cellular homeostatic functions. Thus, the deep comprehension of how NF- $\kappa$ B pathways are modulated during *M. tuberculosis* infection is critical to delineate better intervention strategies pursuing the optimization of disease control.

### Author Contributions

EPA and BBA designed the research, performed the literature search and wrote the manuscript.

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