Clinical & Translational Immunology

Australian and New Zealand Society for Immunology Inc.

Clinical & Translational Immunology 2020; e1179. doi: 10.1002/cti2.1179
www.wileyonlinelibrary.com/journal/cti

REVIEW

March of *Mycobacterium*: miRNAs intercept host cell CD40 signalling

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Received 5 May 2020;
Revised 22 August 2020;
Accepted 22 August 2020

doi: 10.1002/cti2.1179

Clinical & Translational Immunology
2020; **9**: e1179

Abstract

The disease tuberculosis is fatal if untreated. It is caused by the acid-fast bacilli *Mycobacterium tuberculosis*. *Mycobacterium* resides and replicates within the alveolar macrophages, causing inflammation and granuloma, wherein macrophage-T cell interactions enhance the inflammation-causing pulmonary caseous lesions. The first interactions between *Mycobacterium* and the receptors on macrophages decide the fate of *Mycobacterium* because of phagolysosomal impairments and the expression of several miRNAs, which may regulate CD40 expression on macrophages. While the altered phagolysosomal functions impede antigen presentation to the T cell-expressed antigen receptor, the interactions between the macrophage-expressed CD40 and the T cell-expressed CD40-ligand (CD40L or CD154) provide signals to T cells and *Mycobacterium*-infected macrophages. These two functions significantly influence the resolution or persistence of *Mycobacterium* infection. CD40 controls T-cell polarisation and host-protective immunity by eliciting interleukin-12p40, nitric oxide, reactive oxygen species and IFN-γ production. Indeed, CD40-deficient mice succumb to low-dose aerosol infection with *Mycobacterium* because of deficient interleukin (IL)-12 production leading to impaired IFN-γ-secreting T-cell response. In contrast, despite generating fewer granulomas, the CD40L-deficient mice developed anti-mycobacterial T-cell responses to the levels observed in the wild-type mice. These host-protective responses are significantly subdued by the *Mycobacterium*-infected macrophage produced TGF-β and IL-10, which promote pro-mycobacterial T-cell responses. The CD40-CD40L-induced counteractive immune responses against *Mycobacterium* thus present a conundrum that we explain here with a reconciliatory hypothesis. Experimental validation of the hypothesis will provide a rationale for designing anti-tubercular immunotherapy.

Keywords: anti-mycobacterial T-cell response, CD40-CD40L interaction, mannose receptor, microRNA, *Mycobacterium tuberculosis*, *Mycobacterium*–macrophage interactions

INTRODUCTION

Mycobacterium tuberculosis (*Mtb*) is an acid-fast bacillus that resides and replicates within monocytes and macrophages, in particular, alveolar macrophages (AMs), which play key roles in developing robust innate and adaptive immune responses against the bacterium.[1,2] While the bacterium is eliminated or pushed to dormancy in a resistant host, the pathogen inflicts the disease tuberculosis in a susceptible host. Thus, *M. tuberculosis* infection presents two paradoxes: one, functional duality of the macrophages as a supporting host or as an eliminator, and two, alternate fates of the pathogen in resistant versus susceptible hosts. In principle, the macrophages from the resistant and the susceptible hosts have intrinsic or genetic differences that result in either elimination or growth of *Mycobacteria*.[3,4] Complementary to the intrinsic ability or inability of the host cells to control the infection, the pathogen can suppress the anti-mycobacterial killing mechanisms in the susceptible macrophages. It fails in the resistant macrophages, linking the alternate outcomes of the infection to the virulence of the pathogen.[5] The macrophages are well known to also act as the antigen-presenting cells to the antigen-specific T cells.[6] This innate control of *Mycobacterium* may be linked to the T-cell responses that may further accentuate the initial control of the pathogen. Intracellular signalling regulates three major integrated processes: (1) macrophage–*Mycobacterium* interaction, (2) macrophage–T cell interaction and (3) T-cell regulation of macrophage functions. Here, we analyse these dualities of macrophage functions and alternate outcomes of infection with special reference to CD40-CD40L interaction.

MACROPHAGE–*MYCOBACTERIUM* INTERACTION

Once internalised following multiple ligand–receptor interactions, *Mycobacterium* lives within phagosomal vesicles, which are formed during the phagocytosis of the pathogen. The phagosomes fuse with the lysosomes, which are rich in hydrolytic enzymes, proteases and lipases. The phagosome–lysosome fusion eventuates in the death of the intra-phagolysosomal *Mycobacteria*. The internally degraded antigens are complexed with MHC class I or with MHC class II, and these two classes of antigens are presented to $CD8^+$ or $CD4^+$ T cells, respectively. Once these T cells are activated, the cytotoxic activities of the $CD8^+$ T cells may directly destroy the mycobacterial antigen-expressing macrophages,[7,8] or the cytokines from the activated $CD4^+$ T cells may activate the macrophages to kill the intracellular *Mycobacteria* through reactive nitric oxide.[9–11]

Conventionally, it was believed that cytotoxic activities of $CD8^+$ T cells and cytokine secretion by $CD4^+$ T cells are suppressed in susceptible host exemplifying a scenario, which may be more complex in reality. In many diseases/infections, it has been found that the polyfunctionality and antigenic responses against pathogens are controlled by the metabolic pathways operating in immune cells.[12] T-cell metabolic machinery is regulated in anergy and exhaustion.[13,14] Such mechanisms may be the underlying causes of the lack of optimal anti-bacterial responses as observed in a susceptible host. A resistant host may mount a strong TH1/TH17 response because of the relatively active intracellular metabolic process including glycolysis and upregulation of amino acid transporters SLC1A/EEAT2/GLT-1. Furthermore, the upregulation of CD98 and transferrin receptor can facilitate the cellular energetics positively via activation of the Akt-mTOR axis and control of protein translation of crucial anti-bacterial cytokines and molecules.[15] Indeed, *Mtb*-specific-$CD4^+$ TH1 response moderates protective immunity by producing cytokines such as IFN-γ or TNF-α.[16] CD40L depression strongly correlates with IFN-γ levels in TB patients. In fact, a soluble agonist of CD40L was enough to restore IFN-γ production from PBMCs isolated from TB patients, but not from healthy tuberculin reactor controls, which in turn conjures that defects in CD40L expression in TB patients contribute to diminished levels of IFN-γ.[17] Both interleukin (IL)-12 and IFN-γ productions from human peripheral blood T cells are regulated by mTOR and STAT3.[18] Moreover, the IFN-γ-driven control of *M. tuberculosis* inside infected macrophages requires both iNOS and HIF-1α. Nitric oxide may regulate aerobic glycolysis along with HIF-1α to control intracellular *Mtb* replication.[19] Similarly, in chronic *Mtb* infection, circulating T cells may exhibit an exhausted phenotype characterised by gradual loss of secretion of IL-2 and effectors IFN-γ and TNF-α.[20] The blockade of markers of T-cell exhaustion TIM-3 and PD-1 may restore the functions of TB-

specific $CD4^+CXCR5^+$ T cells.[21] One study describes the presence of such exhausted T cells overexpressing checkpoint marker PD-1 on TH1 cytokine-producing *Mtb*-specific $CD4^+$ T cells in peripheral blood of TB patients. These cells are associated with poor prognosis, and blockade of PD1/PD-L1 checkpoint (using anti-PD-L1 antibodies) can augment the IFN-γ secretion but not the proliferation of $CD4^+$ T cells.[22] By contrast, these processes are suppressed in a susceptible host that results in full-blown disease tuberculosis. The strategies for survival are therefore lined up as soon as *Mycobacteria* attach to the macrophages. One of these strategies is to intercept CD40 expression and function that influences *Mtb* survival or elimination.

Mycobacterium attachment and internalisation

Mycobacterium infection starts with its attachment to the receptors on the macrophage surface and its subsequent internalisation by phagocytosis or receptor-mediated endocytosis aided by opsonisation with serum complements[23] or natural antibodies.[24] Besides, the AM-expressed mannose receptor and surfactant protein A (Sp-A) receptor facilitate endocytosis through recognition of lipoarabinomannan (LAM) and Sp-A on *Mycobacterium*, respectively.[25,26] The scavenger receptors bind the mycobacterial cell wall lipoteichoic acid to enhance the phagocytosis of the bacteria.[27] Besides these receptors, Toll-like receptors (TLRs) are also implicated in the internalisation of *Mycobacteria*. The mycobacterial surface lipoglycoprotein MPT83 and LAM are recognised by TLR2 and TLR4, respectively, to enhance *Mtb* internalisation.[28,29] Dectin-1 ligands that are expressed by *Mtb* await their purification and structural characterisation, as *Mycobacteria* do not express β-1,3-glucans, the known Dectin-1 ligands. Different receptors on macrophages or dendritic cells thus enhance *Mtb* internalisation (Table 1; Figure 1) but exactly how and to what extent these receptors modulate its subsequent intracellular survival remains elusive.

Mycobacterial alteration of host microRNA

Although the mechanism of early *Mycobacterium*–macrophage interaction influencing the subsequent macrophage response is not worked out, the pathogen internalisation is followed by alterations in a huge number of microRNAs (Table 2). In *Mtb*-infected macrophages, miR-23a, miR-125a, miR-146a, miR-579, miR-708, miR-27a, miR-30a, miR-129, miR-1178 and miR-1958 expressions were enhanced, whereas miR-20b and miR-26a expressions were downregulated.[30–52] miR23a modulates TLR2/MyD88/NF-κB signalling to result in enhanced intracellular *Mtb* survival and prevention of macrophage autophagy, as miR-23a inhibitors attenuated *Mtb* survival but enhanced autophagy.[30] Mycobacterial surface sulfoglycolipids act as competitive antagonists of TLR2 and inhibit NF-κB activation to impair cytokine production or costimulatory molecule expression.[31] Similarly, *Mtb* lipoproteins LprG, the glycolipid phosphatidylinositol mannoside-6, and the lipoglycan lipomannan bind TLR2 to induce ERK-1/2-dependent TNF-α production in macrophages.[32] In *Mtb*-infected macrophages, TLR4-enhanced miR-125a directly targets TRAF6 negatively regulating NF-κB to suppress cytokines, attenuate immune response, and promote mycobacterial survival.[33,34] Apparently, miR-708 supported *Mycobacterium* survival and inflammatory response.[35] miR-579 downregulated its mRNA targets – SIRT1 and PDK1 – to enhance macrophage apoptosis and death[36] in human macrophages. miR-1178 overexpression enhanced the intracellular growth of *Mycobacteria* but attenuated the accumulation of IFN-γ, IL-6, IL-1β and TNF-α, while miR-1178 knockdown suppressed the *Mycobacteria* survival and enhanced the expression of these pro-inflammatory cytokines in human macrophages.[42] The TLR2/MyD88/NF-κB signalling-induced miR-27b expression suppressed the NF-κB-mediated induction of pro-inflammatory factors but increased p53-dependent production of reactive oxygen species and bactericidal functions of macrophages.[43] miR-26b negatively regulated the NF-κB pathway by directly targeting TGF-β-activated kinase-1 (TAK1), resulting in inhibition of immune response, and promotion of *Mtb* replication and gene expression.[44] miR-106b targeted the 3′-UTR of Cathepsin S resulting in its silencing and impaired antigen processing by the *Mtb*-infected macrophages.[45] Similar regulations were observed with miR-20b in tuberculosis patients and *M. tuberculosis*-infected mice.[46] During *Mtb* infection, miR-26a facilitated arginase activity but reduced iNOS activity,[47] and iNOS expression was also reduced by miR-146a by the inhibition of TRAF6, p38MAPK and NF-κB.[48] miR-26a directly

Table 1. Receptors mediating the internalisation of *Mycobacterium tuberculosis*

No.	Receptors expressed by host cells	Ligand binding mechanism	Implication
1.	CD14 receptors	The entry of nonopsonised tubercle bacilli into brain microglia	Promoting TNF-α production
2.	CR1 (CR1, CD35)	Binding to complement fragments C3b/C4b deposited on *mycobacteria*	Licensing entry inside macrophages
3.	CR3 (CD11b, CD18)	Opsonised *Mycobacterium tuberculosis* binds CR3 at its iC3b binding domain; Nonopsonised *Mycobacterium tuberculosis* uses its endogenous capsular polysaccharides to interact with the β-glucan binding site near the C terminus of CD11b	Uptake of complement opsonised bacterium and activating the alternative complement pathway
4.	CR4 (CD11c, CD18)	*Mycobacterium tuberculosis* macrophage binding in the absence of serum	Tyrosine phosphorylation of a major 60-kDa protein in host cells ($p60^{src}$)
5.	DC-SIGN	Binding with LAM	Potentiate TLR-4-mediated IL-10 secretion by LPS-stimulated MoDCs
6.	Dectin-1	Binding with an unknown ligand on *Mtb*	Promoting mycobacterial-induced IL-12p40 production by DC
7.	Fcγ receptors	Immunoglobulin G (IgG)-mediated opsonisation of *Mtb* bacilli	Redirecting intracellular trafficking of *Mtb* containing vesicles with ferritin-loaded lysosomes
8.	Fibronectin (Fn)	The interaction may occur through the binding of bacterial fibronectin-binding proteins (FnBPs) with fibronectin	Dispensable for *Mtb* attachment and internalisation
9.	Mannose Receptor (CD206)	LAM mediated binding to MR1ManLAM inhibits phagosome maturation	Synthesis of IL-10, IL-1R; inhibiting IL-12 production; blocking of phagosome maturation
10.	Mincle	Recognition of *Mtb* ligand glycolate trehalose dimycolate	Mincle-mediated secretion of inflammatory cytokines/chemokines and promotion of granuloma
11.	Scavenger receptor class A (MARCO)	Interaction with bacterial cell wall components and LDL	*Mtb* 'tether' cell wall glycolipid, trehalose 6,6′-dimycolate TDM/Cord factor to the macrophage and to activate the TLR2 signalling pathway
12.	Scavenger receptor class B (SR-B1/ CD36)	ManLAM and LM; diglycerides lipoteichoic acid (LTA)	Facilitating the availability of lipoproteins to TLR2 heterodimers
13.	SIGNR3	LM and ManLAM; lipoprotein LpqH	SIGNR3 can 'collaborate' with TLR2 for inducing pro-inflammatory cytokine secretion
14.	Surfactant protein A (Sp-A)	*Mtb* binding to SP-A is dependent on calcium and glycosylation of Sp-A	It enhances binding and phagocytosis of *Mtb*
15.	Surfactant protein D (Sp-D)	SP-D through its carbohydrate recognition domain binds to the terminal mannose caps of LAM	Agglutination; Reducing phagocytic uptake; increasing PL fusion
16.	TLR2	Reported *Mtb* ligands for TLR2: LAM, LM, PIM, and lipoglycan binding; lipoproteins LpqH and LprG; Rv0577 and hsp70; PE_PGRS33	ERK1/2 phosphorylation and TNFα production; macrophage apoptosis, consequently promoting containment of *Mtb*
17.	TLR4	*Mtb* 50S ribosomal protein Rv0652; H37Rv	Inducing IRF3 to encourage IFN-β secretion
18.	TLR9 (Intracellular)	Undermethylated CG motifs (CpG) within bacterial DNA	Inducing IL-12p40 and TNF-α production

targeted the transcription factor KLF4 to prevent lysosomal trafficking of *Mtb* and to regulate *Mtb* survival in macrophages.[47] Enhanced miR-155 expression in *Mtb*-infected macrophages suppressed the lipidation and autophagosome formation in dendritic cells enhancing mycobacterial survival.[49] While studying a network of 77 putative miRNAs in early *Mtb*-infected macrophages, miR-155 was found to exhibit dual roles in the survival of the *Mtb*-infected macrophages and the *Mtb*-specific T cells through SHIP-1/protein kinase B (Akt) pathway.[50] On the one hand, miR-155 generated a favorable niche for the pathogen, and on the other hand, it enabled an effective adaptive immune response.[49–51] Similarly, *Mtb*-induced miR-33 is

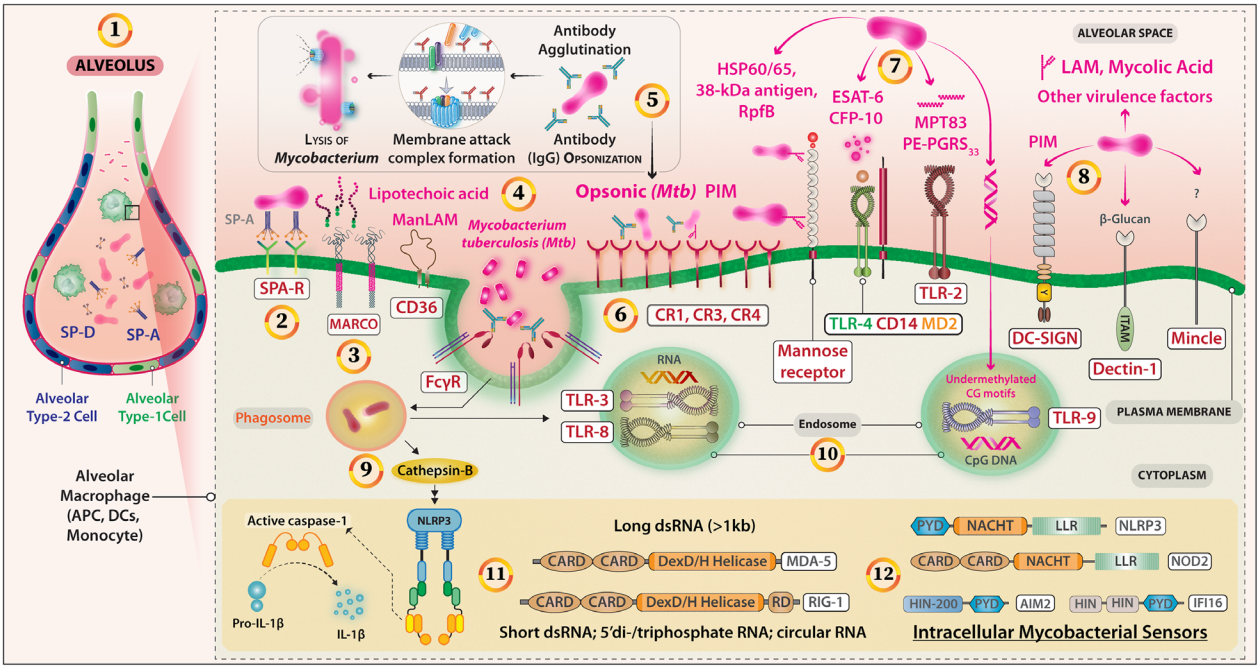

Figure 1. Receptors implicated in the internalisation of *Mycobacterium* sp. and intracellular sensors. **(1)** Pulmonary *Mycobacterium* infection begins with the bacilli entering into the airway where airway epithelial cells (AEC) respond by synthesising antimicrobial peptides (AMPs) and proteins, for example, Collectins Surfactant protein A (SP-A) and SP-D proteins. **(2)** These proteins opsonise the bacteria and facilitate phagocytic uptake by alveolar macrophages through SP-A receptors. **(3)** Mycolic acid and lipoteichoic acid in the *Mtb* cell membrane play distinct roles in receptor-mediated internalisation. Lipoteichoic acid binds to the MARCO (Class-A scavenger receptor) and affects cytokine production in a TLR2-dependent manner. **(4)** The entry of *Mycobacterium* is also supported by an array of pattern recognition receptors (PRRs) including, Toll-Like receptors (TLRs) TLR4-CD14, TLR2, Mannose receptors, immunoglobulin G (IgG)-coated *Mycobacteria* via FcγRs and Scavenger receptors that bind lipopolysaccharides of gram-negative bacteria and lipoteichoic acid of gram-positive bacteria. **(5)** The abundance of C3 and C3bi proteins in broncho-alveolar lavage fluid marks the *Mycobacterium* for complement-mediated lysis through the alternative pathway. This binding also enhances its phagocytic uptake via complement receptors expressed by alveolar APCs. **(6)** Serum-derived ligands facilitate pathogen uptake via receptors CR1, CR3 and CR4 and translocated to membrane-bound phagosomes. CR3 through its interaction with Mannosyl-phosphatidyl-Myo-inositol-based glycolipids (PIM) can also facilitate *Mycobacterium* uptake. **(7)** A battery of major virulence proteins from mycobacterial species are reported. Lipoarabinomannan (LAM) and mannosylated LAM (ManLAM) are two major representatives. Others include HSP60/65, 38kDa protein/Ag38, *Mtb* resuscitation-promoting factor (RpfB), ESAT-6, CFP-10, MPT83, PE-PGRS$_{33,}$ and these factors have different mechanisms of binding to the host cells. **(8)** The C-type lectin and DC-Sign receptors (CD209) mediate *Mtb* entry via binding to PIM similarly. Other CLRs are Mincle and Dectin-1 that may also mediate the internalisation process through unknown mechanisms. Intracellular Sensors: **(9)** During *Mtb* infection, the lysosomal release of cathepsin B (CTS-B) plays an important role in NLRP3-inflammasome activation and subsequent rise in IL-1β production. This pathway may also control pyroptosis. **(10)** TLR3 and TLR8 are activated by mycobacterial RNA while undermethylated CpG motifs from the *Mtb* genome may activate TLR9 to elicit the cytokine biosynthesis. **(11)** Many other intracellular sensors of mycobacterial moieties have been reported. These include RIG-1, MDA-5 and PKR that may contribute to the upregulation of Type-I IFNs. NOD2 activation may occur through GMDP which is a metabolite of the *Mtb* cell wall. **(12)** Cytosolic DNA sensor AIM-2 mediates IL-1β and IL-18 production in response to *Mtb*. IFI16 is an innate immune sensor for intracellular DNA that may lead to the activation of the cytosolic surveillance pathway (CSP).

reported to modulate an array of responses in the host ranging from autophagy, lysosomal function and fatty acid oxidation to support *Mtb* replication.[52] These observations suggest that during the early interaction with TLRs and other receptors on host macrophages, *Mtb* triggers intricate intracellular signalling pathways that selectively regulate the miRNAs that counteractively control *Mtb* fates in macrophage (Figure 2). The miRNA manipulated phagolysosomal compartment affects antigen processing and presentation influencing the T-cell responses. It remains to be investigated whether the miRNAs show kinetic regulation of their expression to match the requirements of the immune system to mount a host-protective immune response. Dissection of the miRNAs specificity for intracellular signalling, accompanied

Table 2. *Mycobacterium* infection results in alterations in a large number of microRNAs within the host cells these up- and down-regulations are highly dependent upon the infecting Mycobacterial species and responding host cell types to produce context-dependent cellular effects

miRNA	Status[a]	Targets	Cell types	*Mycobacterium* species	Comment/mechanism(s) of action
Undetermined					
1. miR-125b	–	κB-Ras2 3′UTR	Primary human mϕ	–	Estradiol represses NF-κB activation through induction of κB-Ras2
2. miR-129	–	SP3	Predicted	*Mycobacterium tuberculosis*	SP3 maintains M1/M2 plasticity
3. miR-150↓;miR-485-3p↑	–	–	THP-1	*Mtb Beijing/W, non-Beijing/W clinical strains*	Alterations in the Wnt pathway, insulin pathway, TGF-β pathway, and glycosaminoglycan biosynthesis
4. miR-33	–	NOD2	Predicted	*Mycobacterium tuberculosis*	Downregulation of NOD2 dampens the inflammatory response
5. miR-365	–	IL-6	HEK293	–	Post-transcriptional level regulation of IL-6 by miRNA-365
6. miR-455-5p	–	SOCS3	Predicted	*Mycobacterium tuberculosis*	The expression of miR-455-3p downregulate SOCS3 expression which promotes M2 phenotype
7. Sp110	–	miR-125a; miR-146a; miR-155; miR-21a; miR-99b	RAW264.7	*Mycobacterium tuberculosis H37Ra*	Sp110-mediated macrophage resistance to *Mtb* underlines the inhibiting of multiple miRNAs and modulating host immune response
Upregulated					
8. hsa-let-7b-5p	↑	APO-1/FAS/CD95	THP-1	*Mycobacterium tuberculosis*	hsa-let-7b-5p helps intracellular survival of *Mtb* in THP-1 cells by downregulating Fas protein level
9. hsa-miR-144-5p	↑	DRAM2	TB patients; PBCs; Tissues	*Mycobacterium tuberculosis*	Inhibiting anti-bacterial autophagy
10. miR let-7e	↑	CASP3	Human MDMs	*Mycobacterium avium*	Interfering with the regulation of apoptosis
11. miR-106b-5p	↑	Cathepsin S	Human mϕ	*Mycobacterium tuberculosis*	*Mtb* avoids exposure to degradative enzymes in the endocytic pathway
12. miR-1178	↑	TLR4	Human mϕ; HTP-1; U937 cells	*Mycobacterium tuberculosis*	Reduction of pro-inflammatory cytokines- IFN-γ, IL-6, IL-1β, and TNF-α
13. miR-124	↑	MyD88; TRAF6;TLR6	TB patient Leucocytes; RAW264.7 AM	*Mycobacterium tuberculosis; Mycobacterium bovis (BCG)*	Negative regulatory role of miR-124 in the fine-tuning inflammatory response in alveolar macrophages
14. miR-125a	↑	UVRAG	RAW264.7; J774A.1	*Mycobacterium tuberculosis*	Inhibiting autophagosome formation thereby promoting intracellular growth of *Mycobacterium tuberculosis*
15. miR-129-3p	↑	Atg4b	RAW264.7	*Mycobacterium tuberculosis*	Inhibiting autophagy favors *Mtb* survival
16. miR-132;miR-26a	↑	p300	Primary human mϕ	*Mycobacterium tuberculosis*	Limiting macrophage responses to IFN-γ
17. miR-140	↑	TRAF6	TB patient PBMCs; THP-1 and U937	*Mycobacterium tuberculosis*	miR-140 promotes *Mtb* survival by suppressing pro-inflammatory cytokines production
18. miR-142-3p	↑	N-Wasp	J774A.1; Primary Human mϕ	*Mycobacterium smegmatis*	Alterations of actin filament assembly affecting other early events of phagolysosome biogenesis
19. miR-143;miR-365	↑	c-Maf, Bach-1, and Elmo-1	BMDMs	*Mtb clinical Beijing strain HN878*	miRNA-mediated regulation of c-Maf, Bach-1, and Elmo-1 in *Mtb*-infected (IL-4/IL-13) macrophages
20. miR-144	↑	-	TB patients PBMCs	*Mycobacterium tuberculosis*	Inhibiting TNF-α and IFN-γ production and T-cell proliferation
21. miR-144-3p	↑	ATG4a	RAW264.7	*Mycobacterium bovis (BCG) Mycobacterium tuberculosis*	Inhibiting the formation of autophagosomes.
22. miR-145	↑	TIRAP	MDMs	*Virulent H37Rv*	Elicited only by virulent H37Rv infection

(Continued)

Table 2. Continued.

miRNA	Status[a]	Targets	Cell types	*Mycobacterium* species	Comment/mechanism(s) of action
23. miR-146	↑	IRAK1; TGFBR2	Bovine mϕ cell line (Bomac)	*Mycobacterium bovis*	Post-transcriptional regulation of IL-1, TLR signalling via IRAK1
24. miR-146a	↑	TRAF6	RAW264.7; BMDMs	*Mycobacterium tuberculosis*	Suppressing nitric oxide production via iNOS
25. miR-146a	↑	–	Human PBMCs	*Mycobacterium abscessus*	*Mycobacterium abscessus* may promote a neutrophil-dependent growth niche
26. miR-155	↑	ATG3	Human DC	*Mycobacterium tuberculosis*	Subverting autophagy
27. miR-155	↑	SHIP1	Mϕ	*Mycobacterium tuberculosisMycobacterium bovis (BCG)*	miR-155 regulating macrophage survival and T-cell expansion through SHIP1; miR-155 repressing the expression of SHIP1 and modulating ROS production
28. miR-155	↑	Rheb	BMDMs; RAW264.7	*Mycobacterium tuberculosis*	Induction of miR-155, in turn, activates autophagy by targeting Rheb
29. miR-155	↑	FOXO3	THP-1; TB Patients PBMCs	*Mycobacterium tuberculosis*	Apoptosis inhibition through regulating FOXO3 target genes
30. miR-155;miR- 31	↑	PP2A (Ppp2r5a)	RAW264.7; BMDMs	*Mycobacterium bovis (BCG)*	*Mycobacterium bovis BCG*-induced miR-155 and miR- 31 are required for activating the WNT-SHH pathway and autophagy regulation
31. miR-1958	↑	Atg5	RAW264.7	*Mycobacterium tuberculosis*	Inhibiting autophagy by interacting with Atg5 and supporting intracellular *Mtb* survival
32. miR-199a	↑	TBK1	J774A.1; BMDM	*Mycobacterium bovis*	Suppressing maturation of autophagosomes and interferon-β (IFN-β) production
33. miR-206	↑	TIMP-3	THP-1	*Mycobacterium tuberculosis*	miR-206 is a regulator of inflammation and MMP-9 by targeting TIMP3
34. miR-21	↑	PFK-M	BMDM; Human MDM; RAW267.4	*Mycobacterium tuberculosisH37Ra, H37Rv*	*Mtb* limits glycolysis in host macrophages through sustained induction of anti-inflammatory miR-21
35. miR-21	↑	IL-12p35; Bcl-2	BMDMs; BMDCs	*Mycobacterium bovis (BCG)*	Modulating anti-mycobacterial TH1 response inefficacy of BCG vaccination
36. miR-223	↑	CXCL2; CCL3; IL-6	TB patients; Murine myeloid cells	*Mycobacterium tuberculosis*	miR-223 regulating leucocyte chemotaxis via chemoattractants
37. miR-22-3p	↑	Unknown	TB patient	*Mycobacterium tuberculosis*	Plasma biomarker
38. miR-23a-5p	↑	TLR2	RAW264.7	*Mycobacterium tuberculosis*	Modulation of TLR2/MyD88/NF-κB signalling
39. miR-27a	↑	CACNA2D3	Human PBMCs	*Mycobacterium tuberculosis*	Inhibiting autophagosome formation and promoting the intracellular survival of *Mtb*
40. miR-27b	↑	Bag2	RAW264.7; HEK293T	*Mycobacterium tuberculosis*	miR-27b positively regulates apoptosis by directly targeting Bag2 and increasing the activity of the p53–ROS signalling pathway
41. miR-29a	↑	CASP7	Human MDMs	*Mycobacterium avium*	Interfering with the regulation of apoptosis
42. miR-30a	↑	MyD88	THP-1 cells	*Mycobacterium tuberculosis* H37Rv	Inhibiting TLR/MyD88 activation and cytokine (TNF-α, IL-6, IL-8) expression
43. miR-31;miR-150	↑	MyD88	TB patients PBMCs; BMDMs	*Mycobacterium bovis (BCG)*	Sonic hedgehog signalling-responsive miR-31 and miR-150 target MyD88 suppressing TLR2 signalling

(Continued)

Table 2. Continued.

miRNA	Status[a]	Targets	Cell types	*Mycobacterium* species	Comment/mechanism(s) of action
44. miR-3178	↑	TRAF-3	THP-1	*Mycobacterium tuberculosis H37Rv*	More research is required for inferring definitive roles of this miRNA in the context of *Mtb* infection
45. miR-32-5p	↑	FSTL1	THP-1 and U937	*Mycobacterium tuberculosis*	TLR-4/miRNA-32-5p/FSTL1 axis modulating host defence against mycobacterial infection
46. miR-33	↑	ABCA1; ATG5; LAMP1	THP-1	*Mycobacterium tuberculosis*	Target's autophagy suppression, and compromisation of lysosomal function, and lipid homeostasis
47. miR-3619-5p	↑	Cathepsin S (CTSS)	THP-1	*Mycobacterium bovis (BCG)*	CTSS targeting by miR-3619-5p impairs the degradation of autophagic substrates thus blocking autophagosome-lysosome processing
48. miR-381-3p	↑	CD1c	TB patient DCs	*Bacillus calmette-Guérin (BCG)*	Suppression of lipid antigen presentation and induction of IL-10
49. miR-579	↑	SIRT1; PDK1	Human mϕ	*Mycobacterium tuberculosis*	Macrophage cell death and apoptosis
50. miR--5p	↑	Bcl-2; TLR4	RAW264.7 and THP-1	*Mycobacterium tuberculosis*	Enhances *Mtb* survival and apoptosis, by attenuating the secretion of inflammatory cytokines (IL-1β, IL-6, and TNF-α)
51. miR-708-5p	↑	TLR4	Human mϕ	*Mycobacterium tuberculosis*	Reduction of pro-inflammatory cytokines- IFN-γ, IL-6, IL-1β, and TNF-α
52. miR-889	↑	TWEAK	Latent TB patients	*Mycobacterium tuberculosis*	miR-889 inhibits autophagy via suppression of TWEAK expression
53. miR-99b	↑	TNFRSF4/OX40; TNF-α	DC and mϕ	*Mycobacterium tuberculosis* H37Rv	The knockdown of miR-99b in DCs reduces *Mtb* growth owing to increasing levels of IL-1β, TNF-α
Downregulated					
54. miR let-7f	↓	A20/TNFAIP3	RAW264.7	*Mycobacterium tuberculosis*	*Mycobacterium tuberculosis* macrophage infection leads to ESAT-6-dependent miRNA let-7f downregulation
55. miR-125b	↓	TNF mRNA	Human mϕ	*Mycobacterium smegmatis*	TLR2-dependent MAPK p38 and the PI3K/Akt pathway with the production of steady-state TNF mRNA
56. miR-144	↓	Tpl2/MAP3K8	MDMs	*Mycobacterium tuberculosis*	Suppression of TNF-α, IL-1β, and IL-6 via the ERK1/2 pathway
57. miR-17	↓	ULK1; Beclin 1; ATG7; MCL-1 ATG16L1; p62; STAT3	RAW264.7	*Mycobacterium tuberculosis*	miR-17/PKC δ/STAT3 axis regulates autophagy during *Mtb* infection
58. miR-20b	↓	NLRP3	TB patient mϕ	*Mycobacterium tuberculosis*	Deactivating the NLRP3/caspase-1/IL-1β pathway in TB mice; Mitigating the inflammation and pyroptosis
59. miR-20b-5p	↓	Mcl-1	RAW264.7	*Mycobacterium tuberculosis*	Enhancing *Mtb* survival via attenuating the cell apoptosis by Mcl-1 upregulation
60. miR-26a	↓	KLF4	RAW264.7	*Mycobacterium tuberculosis*	Facilitates upregulation of KLF4 consequently increases arginase and decreases iNOS activity; affecting the trafficking of *Mtb* to lysosomes
61. miR-26b	↓	TAK-1	THP-1	*Mycobacterium tuberculosis*	miR-26b suppresses the TNFα-induced NF-κB signalling in THP-1 cells
62. miR-27a	↓	TAB 2/3	RAW264.7; BMDMs	*Mycobacterium avium* subspecies *paratuberculosis*	Inhibiting the activation of the MAPK-p38 signalling

(Continued)

Table 2. Continued.

miRNA	Status[a]	Targets	Cell types	*Mycobacterium* species	Comment/mechanism(s) of action
63. miR-27a	↓	IRAK4	THP-1	*Mycobacterium tuberculosis*	miR-27a inhibiting the release of inflammatory factors and promoting mycobacterial survival
64. miR-29	↓	IFN-γ mRNA	NK cell; $CD4^+$; $CD8^+$ T cells	*Mycobacterium bovis (BCG)*	miR-29 suppresses IFN-γ production by directly targeting IFN-γ mRNA
65. miR-3178	↓	TRAF-3	GES-1 cells	*Helicobacter pylori*	Contrasting to *Mtb* infection, miR-3178 is downregulated, controls inflammation, and gastric carcinogenesis in this model

[a]Status of miRNAs indicating Downregulation (↓) and Upregulation (↑)

by kinetic regulation of each of those miRNAs, will lead to the scientific rationale for a plausible miRNA based anti-mycobacterial therapy.

miRNA regulation of CD40 expression in *M. tuberculosis*-infected macrophages and dendritic cells

An effective anti-mycobacterial therapy would require appropriate T-cell response, which is dependent on CD40-CD40L interactions. While CD40 signals through many signalling pathways in macrophages,[53] signalling through CD40L potentiates the T-cell antigen-specific receptor-activated T-cell functions.[54] It is reported that several microRNAs regulate CD40 expression in various cell types. For example, miR-145 down-regulates CD40 expression specifically in vascular smooth muscle cells[55] and in human monocyte-derived macrophages.[56] TNF-α increases CD40 expression in a model of atherosclerosis but reduce miR-145 expression.[57] miR-146a targets TRAF6 and IRAK1 to repress CD40 expression in PBMCs obtained from patients with myasthenia gravis[58] and perhaps also in other cell types.

IFN-γ and TNF-α – the cytokines that activate macrophages to kill *Mtb* – are shown to enhance CD40 expression. In *Mtb*-infected macrophages, IFN-γ that inhibits miR-21 enhances CD40 expression and anti-mycobacterial functions.[59,60] Opposing IFN-γ and TNF-α, transforming growth factor-β (TGF-β) deactivates macrophages to impair anti-mycobacterial functions and reduces CD40 expression in macrophages.[60] miR-21 thus inhibits TNF-α-induced CD40 expression via the SIRT1-NF-κB signalling pathway.[61] IFN-γ activates STAT-1 homodimerisation to execute its effects. *Mtb* upregulates expression of miR146a that targets STAT1 to reduce CD40 expression.[62] miR-29a augments CD40 expression in bone marrow-derived DCs.[63] While miR-29a targeted IFN-γ mRNA reduces its expression, IFN-γ reciprocally inhibited miR-29a expression in T cells.[64] In TB patients, miR-16 is significantly elevated but miR-155 is reduced.[65] While TLR4 stimulation reduces the level of miR-16 that negatively regulates the CD40 expression,[66] *Helicobacter pylori* infection enhances the expression of miR-155 that promotes CD40 and TNF-α expression.[67] Thus, *Mtb* infection modulates the expression of miR-16, miR-21, miR-29a, miR-145, miR-146a and miR-155, which in turn regulate CD40 expression (Figure 3).

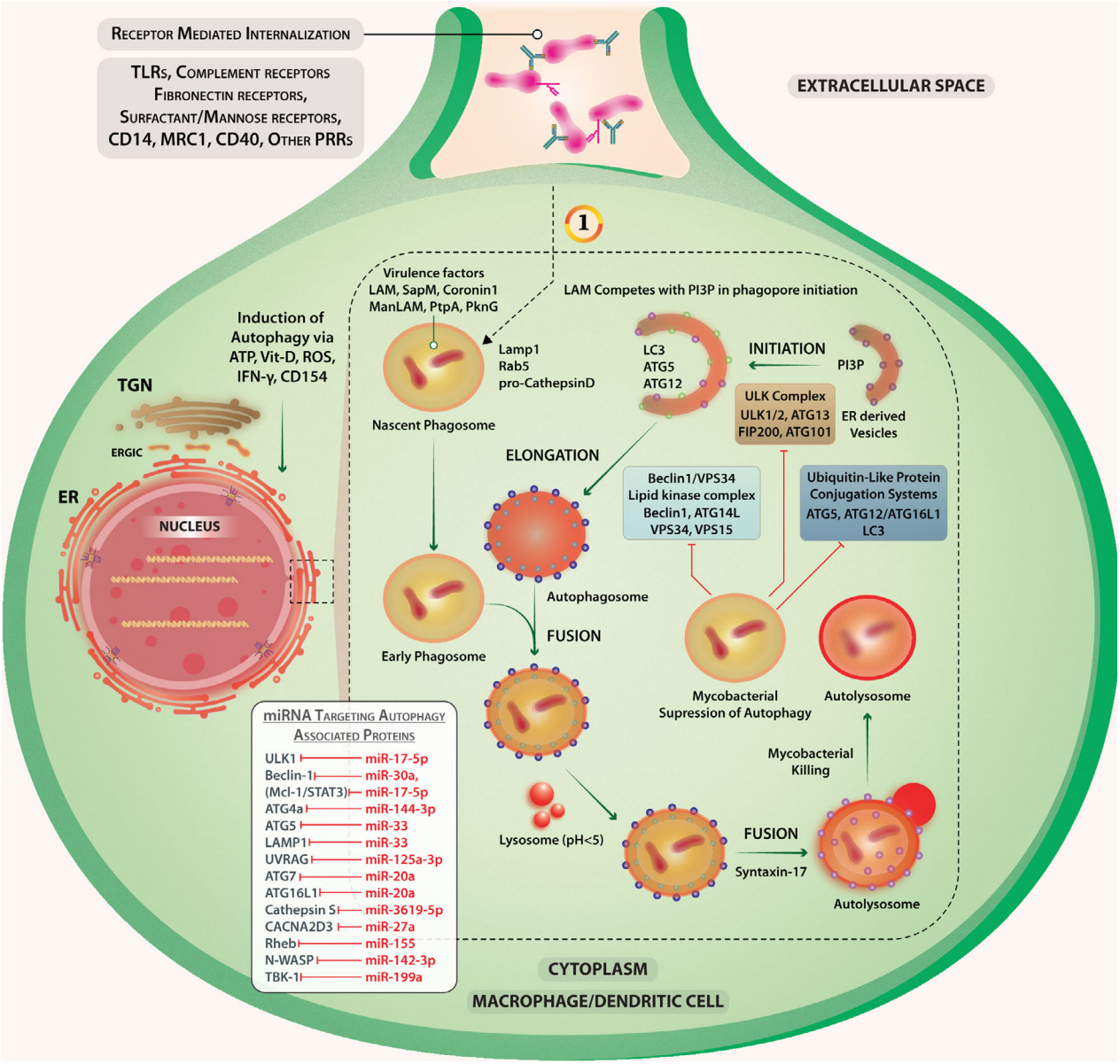

Figure 2. Regulatory miRNAs network that modulates the process of autophagy and promotes intracellular survival of *Mycobacterium* sp. **(1)** The process of autophagy in *Mtb*-infected macrophages/APCs is shown. *Mycobacterium* modulates the miRNAs by either up- or downregulating the expression of certain miRNAs that have an impact on autophagy and hence its intracellular survival. Many of these miRNAs do influence CD40 expression, CD40 signalling, and subsequent survival or elimination of *Mycobacterium*. However, the use of miRNAs as pathogenic biomarkers for tuberculosis requires consideration of the *Mycobacterium* species and host cell type and genetics of the host. miRNA targeting using antagomiRs–oligonucleotides for devising an anti-mycobacterial strategy seems feasible.

Transcription factors regulate CD40 expression

Besides microRNAs, other factors regulate CD40 expression in macrophages. NF-κB may function as a central regulator of CD40 expression,[68,69] perhaps through TLR4-CD40 and TLR9-CD40 feed-forward motifs as shown in the case of another intra-macrophage pathogen, *Leishmania major*.[70] The mitogen-activated protein kinases (MAPKs) – JNK and p38MAPK but not ERK – may activate NF-κB to augment CD40 expression in both mouse and human macrophages.[71] LPS/TLR4-induced CD40 expression involves the endogenous production of

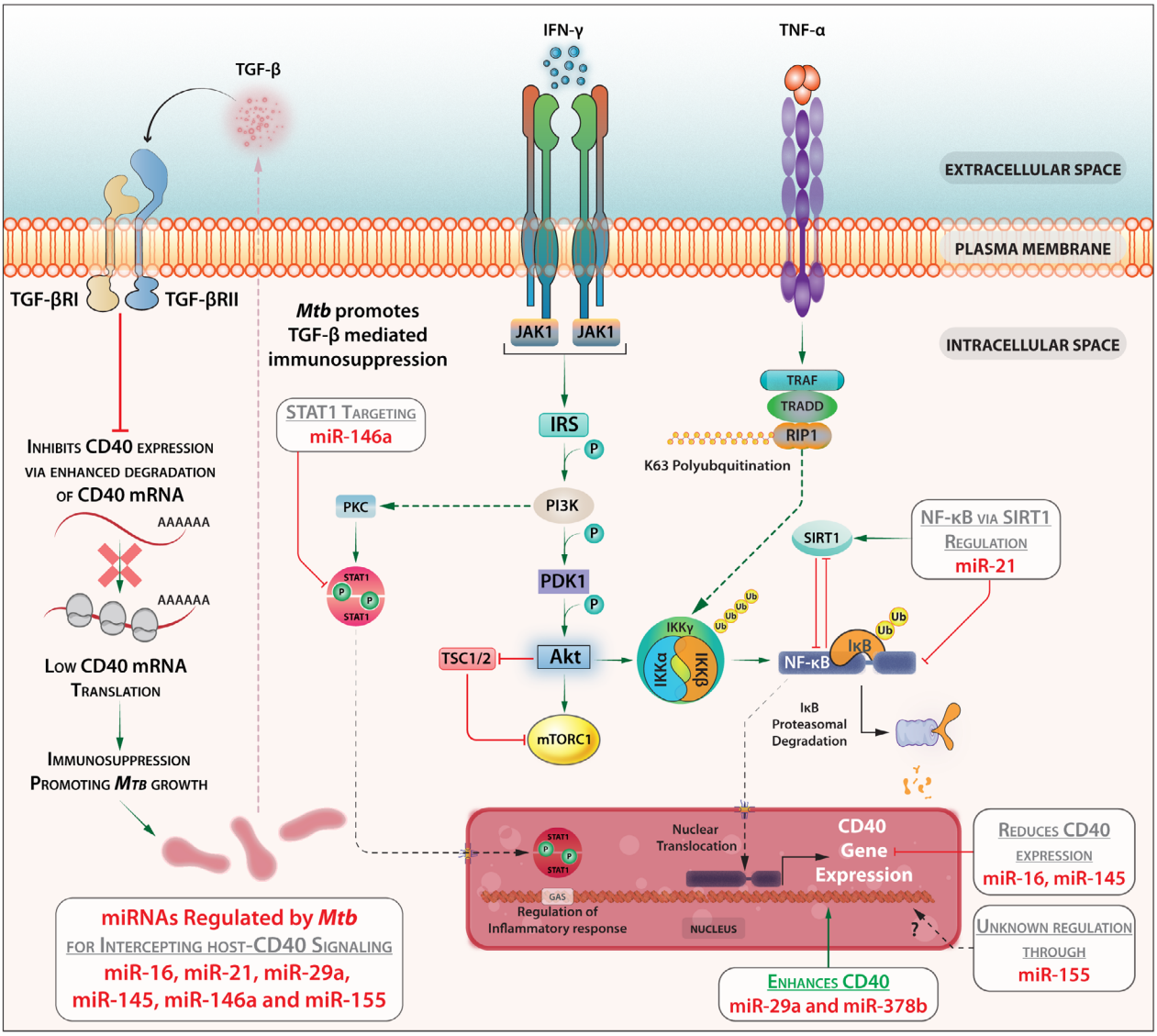

Figure 3. *Mtb* infection modulates the expression of miR-16, miR-21, miR-29a, miR-145, miR-146a and miR-155 which in turn regulate CD40 expression.

the cytokine IFN-β. IFN-β induces not only STAT-1α-dependent CD40 expression but also SOCS-1 that inhibits cytokine signalling affecting CD40 expression in macrophages and microglia. IFN-β-induced CD40 gene expression is thus self-limited by IFN-β-induced SOCS-1 expression.[72] Besides NF-κB, IRF8 is another key transcription factor that regulates CpG-promoted CD40 expression. TRAF6 and IRAK1 may also be targeted by miRNA-146a to reduce CD40 expression in DCs.[73] It is known that the virulent *Mtb* strain H37Rv invades macrophages quicker than the avirulent H37Ra but the avirulent strain induces significantly higher nitric oxide and hydrogen peroxide, IL-12, TNF-α and IFN-γ productions from the infected macrophages. It remains to be investigated whether CD40-CD40L interaction is a key factor in *Mtb* virulence[74,75] and vice versa.

CD40 expression in various circulating and alveolar cells of TB patients

Most of the studies mentioning the roles of miRNAs concerning the modulation of CD40 levels are either performed *in vitro* or using mouse models. Through an exhaustive analysis, Fu *et al.*[76] has

found a huge number of microRNAs in the serum of active pulmonary tuberculosis patients, but it is yet to be determined whether these circulating miRNAs have targets that are involved in CD40 pathways that may produce specific changes in the effector/memory T cells or APCs. However, this proposition also requires experimental validation. We have summarised the roles of such miRNAs (Table 2) in the modulation of CD40-signalling during *Mtb* infection. In general, AMs rely less on glycolysis but more on OXPHOS for meeting their energy requirements under steady-state conditions.[77] AMs exhibit low-efficiency antigen presentation and very low-level expression of costimulatory molecules[78] including CD40. However, infection or other stimulation could enhance the CD40 level among this lung residential APC population.[79] Patients suffering from hyper-IgM syndrome, caused by the mutations in CD40L and thereby defects in CD40 signalling, may have increased susceptibility to intracellular pathogens[80] including *Mycobacterium*.[81]

Although the AMs were traditionally believed to be the only host cell for *Mtb* proliferation, recent findings support that the pathogen could thrive in many different phagocytes within the lung microenvironment. Kinetic studies further defend the concept that the initial distribution of the pathogen remains associated with AMs, but during the chronic phase of infection, the disseminating bacilli and plausibly latent bacteria may spread among other phagocytes including interstitial macrophages perpetuating the infection. This observation supports that diverse macrophage populations in the lungs rather serve as the *Mtb* growth permissive environment in a temporal manner.[82]

Macrophage CD40 expression can be enhanced by IFN-γ through activation of the transcription factors STAT-1 and NF-κB via an autocrine positive feedback loop including IFN-γ-induced TNF-α. IFN-γ-induced CD40 expression is suppressed by antilipidaemic agent simvastatin that inhibits 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase – an enzyme required for the synthesis of isoprenoids and STAT-1 expression. The inhibition of the prenylation of Rho family proteins a family of small GTPases inhibits CD40 and STAT-1 expression. As a consequence, STAT-1α and RNA Polymerase II recruitment to the CD40 promoter are diminished and H3 and H4 histone acetylation is reduced.[83] Functional analysis of CD40 promoter in microglial cells indicates that STAT-1 binds to two IFN-γ-activated sequence elements. The transcription factors PU.1 and/or Spi-B bind to the Ets elements.[84,85] IL-4-activated transcription factor STAT6 binds to these two proximal and distal IFN-γ-activated sequences and represses CD40 expression.[86] Thus, several transcription factors act in tandem to regulate CD40 gene expression in cells of the macrophage lineage (Figure 4).

The induction of CD40-CD40L expression in B cells, DCs and endothelial cells can also be of therapeutic importance. As CD40 engagement on the DCs membrane directly augments the cytokine production, cross-antigen presentation and maturation, CD40 regulates DCs activation and differentiation. Similarly, in the case of B cells, CD40 signalling promotes cell survival, germinal centre formation, Ig class switching and somatic hypermutation of the Ig to enhance Ag affinity and formation of memory and plasma B cells.[87] The involvement of the CD40-CD40L pathway in *Mtb* infection is paradoxical, although targeting this pathway provides long-term clinical benefits in many diseases including organ transplantation[88] and autoimmunity.[89] Similar beneficial effects of CD40-CD40L expression/signalling may constitute a futuristic anti-TB therapy.

Altered antigen processing in *Mtb*-infected macrophages or dendritic cells

Mtb antigen processing is preceded by its uptake into the phagosomal vesicles. One way to survive within the host cells is to stall further maturation of the phagosomes and thereby antigen processing, too.[90] Phagosomal maturation involves fusion with lysosomes (the vesicular organelle rich in hydrolases, proteases, lipases and other enzymes that are required for degradation of the pathogen and the pathogen-derived antigens) so that the resulting peptides can be complexed with MHC class-I or MHC class-II molecules for presentation to T cells as the phagolysosomal vesicles are acidified. *Mtb* inhibits this phagosomal maturation to ensure persistence in the immature phagosomes (Figure 5).

Mtb-secreted EspB [Early Secretory Antigenic Target 6 (ESAT-6) system 1 (ESX-1) secretion-associated protein B)] and EspA suppress antigen-processing functions of the *Mtb*-infected macrophages[91] reduce IFN-γRI expression and inhibit IFN-γ-activated STAT1 phosphorylation.[92,93] Avirulent *Mtb* is perhaps deficient in this system

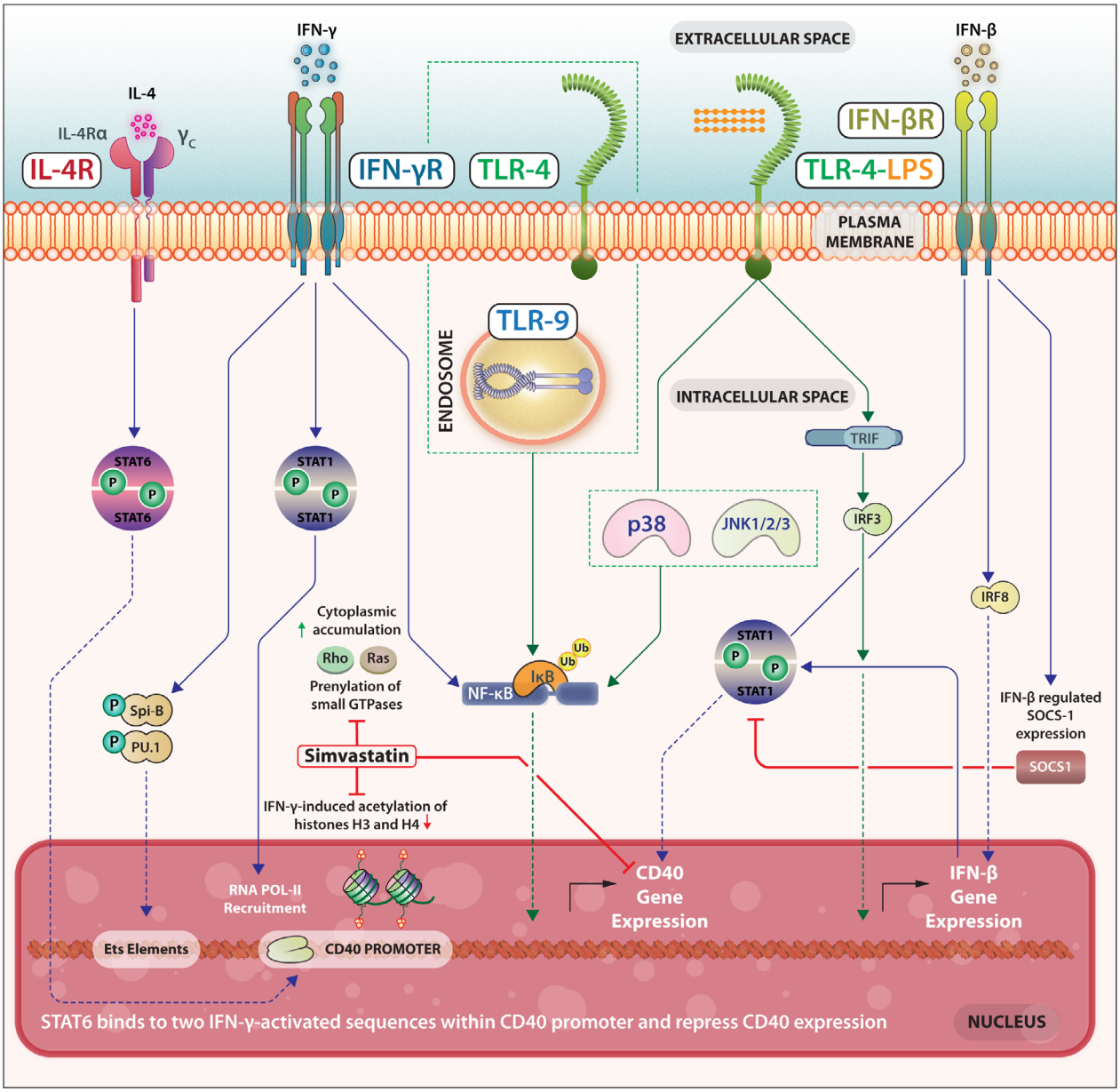

Figure 4. Several transcription factors act in tandem to regulate CD40 gene expression in cells of the macrophage lineage.

and may, therefore, be unable to survive in macrophages. Molecular analyses show that LRRK2 (leucine-rich repeat kinase 2) negatively regulates phagosome maturation via the recruitment of phosphatidylinositol-3 kinase (PI3K) complex and Rubicon to the phagosome in macrophages,[94] as LRRK2 inhibition and LRRK2-deficiency enhance phagosome maturation and significantly reduce *Mtb* burden in macrophages[94] but lysophosphatidylcholine promotes phagosome maturation via cAMP-induced activation of the PKA-PI3K-p38MAPK pathway and controls *Mtb* infection through Ca^{2+} and ROS-dependent pathways.[95] As CD40 also induces the host-protective pathway of PI3K and p38MAPK in macrophages, CD40 stimulation in *Mtb*-infected macrophages would also reduce bacterial burden. CD40 appears to be a likely target of the bacteria,

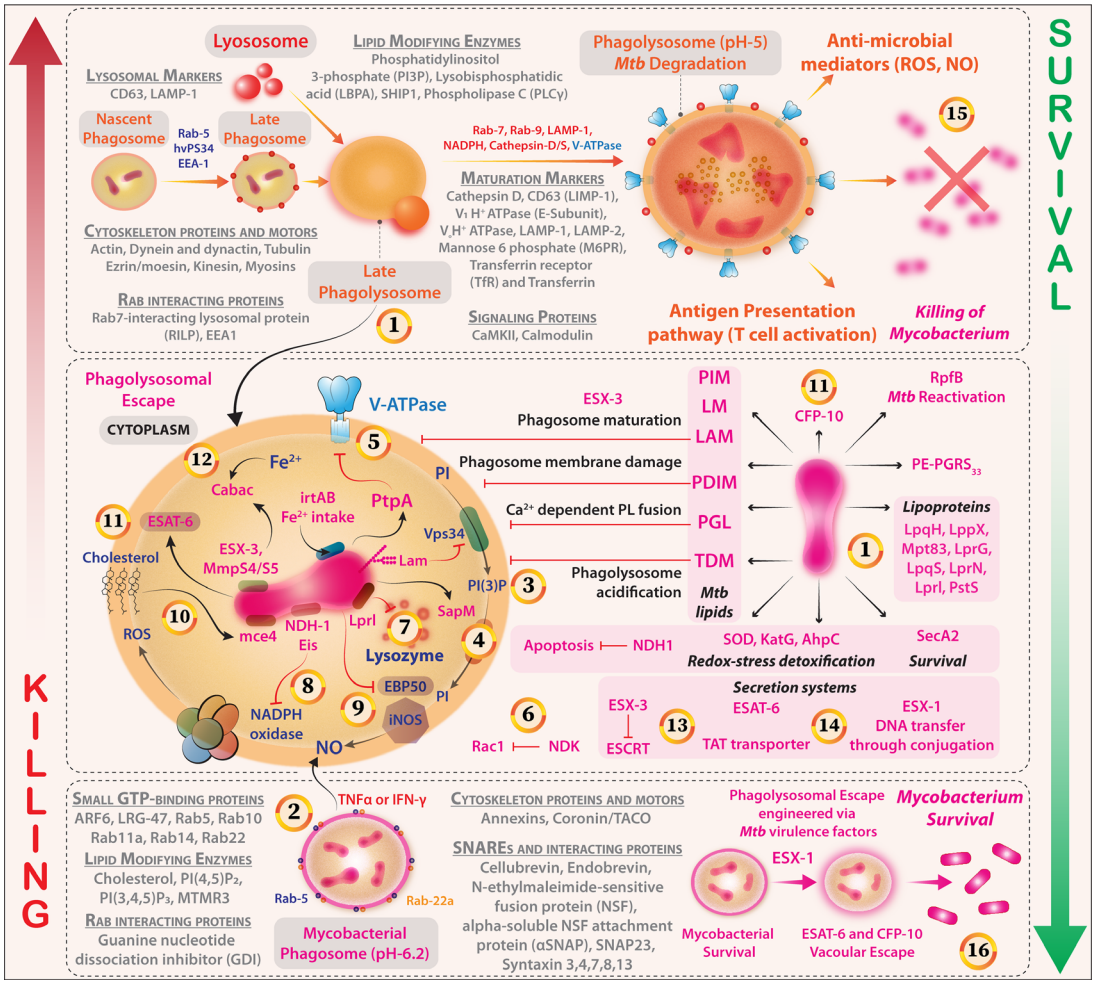

Figure 5. Pathway of phagosome biogenesis, maturation, and phagolysosome fusion for efficient clearance of *Mtb*. Ploys of immunoevasion via *Mtb* virulence factors are also shown. **(1)** *Mycobacterium* deploys several factors that subvert the phagosome biogenesis, maturation and acidification steps that follow its internalisation. Pathogenic *Mycobacteria* reside within compartments devoid of lysosomal contents because of blocking of Ca^{2+} fluxes and receive nutrients through modulation of Rab-dependent vesicular trafficking. LAM and PIM drive these processes. **(2)** Mycobacterial phagosomes (Bottom) through various proteins counter the independent stress factors such as reactive oxygen species (ROS) and reactive nitrogen species (RNS); however, immunological activation with TNFα or IFN-γ results in the maturation of phagosomes by the maturation marker expression and lysosomal fusion (Their distinct markers and associated proteins are represented with Grey Font). **(3)** LAM inhibits Ca^{2+} influx and PI3P-dependent delivery of lysosomal components (V-ATPase and Cathepsin) from the Trans-Golgi network (TGN) to the phagosome. **(4)** *Mycobacterium*, perhaps through secretory acid phosphatase (SapM), targets small GTPases – Rabs, Rhos or ARFs – to affect Coronin-1/TACO-dependent actin cytoskeleton rearrangements and phagosome maturation. **(5)** The mycobacterial protein tyrosine phosphatase (PtpA) inhibits V-ATPase and phagosomal acidification. **(6)** The nucleotide diphosphate kinase (NDK-1) of mycobacterium may inactivate small GTPase Rac-1 and attenuate NADPH oxidase-mediated host protection. **(7)** LprI, a mycobacterial Lipoprotein, inactivates the lysozyme. **(8)** The Type-I NADH dehydrogenase and Eis protein inhibit the NADPH oxidase activity limiting the ROS availability. **(9)** *Mycobacterium* effectively attenuates NO production by interfering with EBP50 and iNOS recruitment. **(10)** The mammalian cell entry protein-Mce4 scavenges cholesterol from host membranes and potentiates lipid body accumulation and mycobacterial survival. **(11)** Early secretory antigenic target-6 (ESAT-6), a major virulence factor that controls NF-κB and interferon-regulatory factors, and CFP-10 engineer vacuolar escape and intracellular survival of *Mycobacterium*. **(12)** *Mtb* hitchhikes intracellular Fe^{2+} stores a major siderophore mediating this process is Carboxymycobactin. **(13)** ESX-3 secretion system (composed of EsxG and EsxH) leads to impairment of ESCRT-mediated endomembrane repair. **(14)** ESX-1 mediates the process of phagosomal to cytosolic translocation. **(15)** A potent phagosomal maturation and intracellular degradation of *Mtb* by the acquisition of indicated markers (Grey fonts). Results in potentiation of APC-T-cell antigenic presentation pathway and confers T cell-based protection against the bacterium. **(16)** In contrast, the association between *Mtb* virulence factors (Factors that are associated with *Mtb* are shown in pink colour) and potent immunosuppression, steps of phagosomal, maturation, acidification, neutralisation/detoxification of redox stress and inhibition of autophagic processes together induce permissive niches for *Mtb* replication and dissemination.

as CD40 expression is reduced in *Mtb*-infected macrophages.

Virulent *Mtb* causes marked disorganisation of actin filaments and F-actin fragmentation in the cytoplasm of infected macrophages, which contributes to delayed phagolysosomal fusion.[96] Mycobacterial polyunsaturated lipids bind ATP and its receptor P2X7 regulating actin polymerisation.[97] cAMP-dependent inhibition of actin polymerisation in phagosomes containing virulent *Mtb* prevents phagolysosomal fusion supporting bacterial growth[98] (Figure 5). Hence, the ability of the lipid/ATP/P2X7 axis to destabilise actin polymerisation and consequently delay phagosome maturation deserves further investigation.

The intravesicular pH in the *Mtb*-inhabited phagosomes is between 6.3 and 6.0, whereas the lysosomal lytic enzymes require a pH lower than 3.0 (Figure 5). Even if these LAMP-1-positive phagosomes fuse with lysosomes, the vacuolar-ATPase that is required for pumping protons into the vesicular lumen is extruded.[99] The impaired acidification associated with vacuolar-ATPase exclusion has negative effects on antigen processing and presentation, as vacuolar-ATPase-dependent phagosomal acidification is necessary for generating processed *Mtb* antigens.[100] The initial *Mtb*–macrophage interaction dictates the state of phagosomal maturation, as TLR2 blockade, but not CR3 blockade, promotes phagosomal acidification and bacterial death[101] (Figure 5).

CD40 AT THE INTERFACE OF MACROPHAGE AND T CELLS

The characteristic caseous lesions in the lung are the sequel of a strong granulomatous response mediated by activated T cells (Figure 6). The T cells are activated by at least two signals: (1) T-cell receptor signal triggered by the recognition of *Mtb* antigens presented by the AMs or dendritic cells in the context of MHC-II or MHC-I molecules and (2) the costimulatory signal from CD28 that interacts with the CD80 and CD86 expressed on the antigen-presenting AMs or dendritic cells. During the macrophage–T cell interaction, the T cell-expressed CD40-ligand (CD40L) binds to the macrophage-expressed CD40 and triggers CD40 signals in the macrophage. CD40 is known to signal through a cascade of kinases to induce NF-κB-dependent IL-12 expression that leads to TH1 cell differentiation and host protection. Additionally, the same CD40 can also signal through a different pathway to generate IL-10 and TGF-β that aggravate the disease by deactivation of macrophages and differentiation of T-reg cells (Figure 6). The antigen-presenting cell-secreted IL-12 works on the T cells through IL-12R to trigger the STAT4-dependent induction of IFN-γ. IFN-γ activates the *Mycobacterium*-infected macrophages to elicit STAT-1-dependent iNOS-catalysed nitric oxide-mediated mycobactericidal functions of macrophages. IL-4, IL-10 and TGF-β antagonise these host-protective functions. Therefore, it is possible that these two counteractive effector functions of CD40-CD40L interaction determine the outcome of *Mtb* infection.

Vaccine-based protection to *Mtb* heavily relies on the induction of IFN-γ-producing $CD4^+$ T cells. IL-17A and IFN-γ are two important cornerstones for vaccine-induced protection against experimental tuberculosis. Through the adoptive transfer of exogenously primed activated DCs into the lungs of vaccinated mice at the time of *Mtb* infection may overcome the lag required for the generation of vaccine-induced memory $CD4^+$ T cells. This effect can be accelerated by the induction of endogenous $CD103^+$ DC and activation of the CD40 pathway through the TLR ligand amph-CpG, coupled with CD40 agonist FGK4.5.[102] Additionally, out of numerous receptor–ligand interactions occurring at the APC-T cell synapses, the CD40-CD154 interaction is vital for the optimal activation of $CD4^+$ T cells. In the case of *Mtb*-infected DCs, their interaction with T cells is required for inducing protective IL-17 response. Blocking the CD40-CD40L interaction with the anti-CD40L antibody MR1 attenuates the IL-17 response to *Mtb*-infected DCs despite stimulation with CD40LT.[103] This effect is also independent of the low *Mtb*-antigenic concentration during the initial phase of infection as observed by others.[104] Therefore, CD40-mediated costimulation may polarise TH17 cells independent of the antigenic loads in airway tissue, which may perhaps be a crucial event in restricting early replication of *Mtb*.[103] These protective effects can also be augmented by signals that are dependent on PRRs as another study advocates that a latency associated protein resuscitation-promoting factor (Rpf) E can induce TLR4-dependent DC maturation and promotes TH1/TH17 type immunity *in vivo*.[105] Our group showed that TLR4 and CD40 can modulate each other's expression in the experimental model of

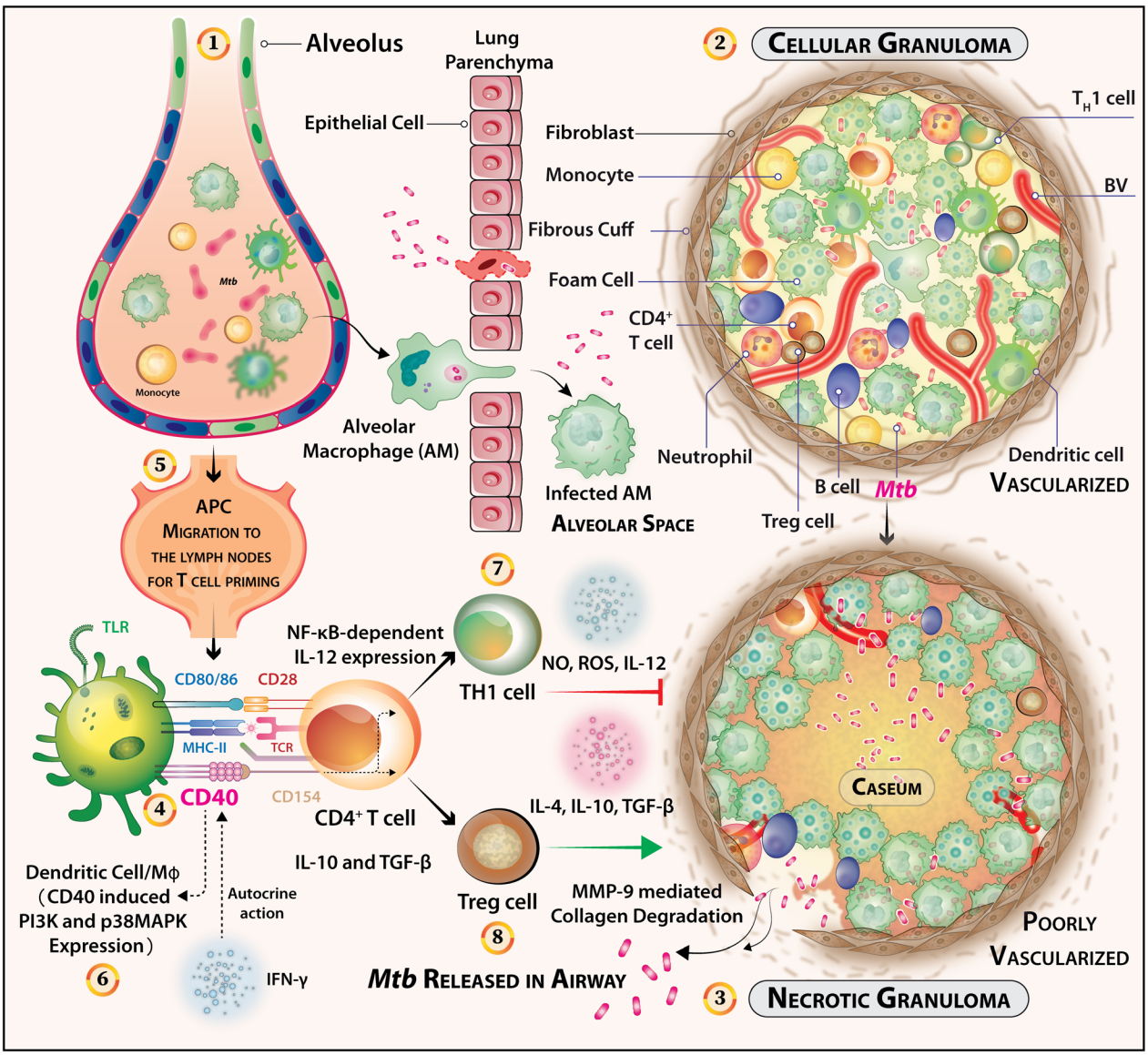

Figure 6. CD40 signals regulate the effector T-cell responses that in turn control the growth of *Mtb,* balancing granuloma pathogenesis and subsequent dissemination of the bacilli in the airway. **(1)** The advent of the tuberculosis disease occurs via confrontation of *Mtb* bacilli and the alveolar macrophages. The infection initiates with receptor-mediated internalisation and triggers a cascade of events that govern the subsequent fates of the pathogen both intracellular and extracellular. **(2)** Infected macrophages may recruit other cell types such as $CD4^+$ T cells, monocytes, neutrophils, B cells and DCs. The granulomatous niche can occur as safe houses for reinitiating latent TB infection. **(3)** However, incapacitated immune responses can lead to the formation of necrotic granulomas indicative of chronic or latent TB infection. These type of granuloma are poorly vascularised and calcified to the core with the characteristic caseous centre. An abundance of foam cells with peripheral fibrotic cuffs abstaining T and B cells can also be marked histologically. Altogether, caseous granulomas can harbour drug-tolerant *Mtb*. **(4)** Nonetheless, the *Mtb* containment strategy of the host can turn on radically upon itself when necrotic granulomas are formed. A strong TH1 cell response may circumvent this critical transition into which receptors like CD40 may have previously unexplored roles. **(5)** Within the draining/thoracic lymph nodes, the T cells are primed slowly at about 12–20 Days post-*Mtb* infection, as indicated in animal models. **(6)** CD40-CD40L crosstalk between T cells, B cells and DCs may promote signals to DCs to induce IL-12 secretion resulting in TH1 cell differentiation and IFN-γ-mediated anti-mycobacterial effects. **(7)** CD40 is known to signal through a cascade of kinases to generate NF-κB-dependent IL-12 expression that leads to host protection by TH1 cells. **(8)** On the contrary, the same CD40 can also signal through a different pathway to generate IL-10 and TGF-β that aggravate the disease by deactivation of macrophages and differentiation of T-reg cells. Therefore, more information is required to dissect the underlying roles of CD40 in mediating the pathogenesis of *Mtb* granulomatous response.

cutaneous leishmaniasis.[70] Possibly, TLR4-CD40 cross-regulation may be controlling the protective immunity against *Mtb* infection.

Presentation of the processed *Mycobacterial* antigens to T cells

The antigen presentation to T cells involves presenting an antigenic peptide in a complex with either MHC class I or MHC class II for recognition by the antigen-specific T-cell receptor (Figure 7). Many of the mycobacterial ligands are elicitors of the cytosolic surveillance pathway (CSP). These pathways are activated by mycobacterial ESX-1 secretion system-mediated extrusion of DNA/RNA allowing activation of host mobile intracellular pathogen sensors including RIG-1, MDA-5, c-GAS/STING/TBK-1, PKR, NLRP3, AIM-2 and others (Figures 1 and 7). The activation of CSP-pathway relates to robust Type-I IFN signatures in response to this pathogen.[106] Although Type-I IFNs may defend against viruses, their induction by bacteria is detrimental to the host.[107]

The number of antigen-loaded MHC molecules and the accessibility of the T-cell receptor to the presented peptide antigen decide the efficacy of this antigen presentation. *Mtb*-infected macrophages express significantly fewer MHC-I and MHC-II molecules on the surface,[108,109] the T-cell receptors' accessibility to the peptide–MHC complex remains to be investigated. TLR2-*Mtb* lipoprotein interaction inhibits IFN-γ-induced MHC-II expression and processing of soluble antigens in a Class II transactivator (CIITA) IV-dependent and MAPK-dependent manner.[110] Repressed MHC-II expression and enhanced TLR2-driven macrophage apoptosis decrease antigen recognition by $CD4^+$ T cells. IL-10 plays a significant role in this process.[111]

Expression of costimulatory molecules on *Mycobacterium*-infected macrophages

The *Mtb*-infected BALB/c-derived macrophages have reduced CD80, but enhanced ICAM-1, expression[112] perhaps mediated by a 10kDa antigen from *Mtb*.[113] Consistent with the enhanced IL-10 production by the *Mtb*-infected macrophages, IL-10 is shown to downregulate the expression of costimulatory molecules on macrophages.[114] As T-cell activation through T-cell antigen receptor in the absence of the costimulatory signal results in T-cell anergy, the antigen presentation by significantly low CD80-expressing *Mtb*-infected macrophages leads to T-cell anergy[115] that has been attributed to IL-10 from the antigen-presenting macrophages.[116] Besides anergy, T-cell response is further reduced by higher levels of PD-L1 expression on *Mtb*-infected macrophages and PD-1 on T cells.[117] CD80-mediated T-cell costimulation is thus balanced by the negative effects of PD1-PD-L1 interaction. However, CD40-CD40L interaction can significantly influence this balance in T-cell response.

CD40-CD40L as a crucial costimulatory receptor–ligand pair in tuberculosis

CD40 signalling, albeit uncharacterised in *Mtb*-infected macrophages, appears to play important roles in eliciting T-cell responses. CD40-CD40L interaction is shown to enhance the IL-12- and IL-18-dependent, CREB- and c-Jun-promoted IFN-γ production by *Mtb*-responsive $CD8^+$ T cells that also execute perforin- and granulysin-mediated cytotoxicity on *Mtb*-infected macrophages.[7] CD40-deficient mice show aggravated *Mtb* infection because of inadequate IL-12 and IFN-γ responses as compared to the wild-type control.[118] An agonistic anti-CD40 antibody elicited strong CD40 signalling in both uninfected and BCG-infected DCs resulting in increased expression of MHC-II and costimulatory molecules, mRNA production related to pro-inflammatory cytokines and IL-12.[119] CD40-deficient *Mtb*-infected, but not the uninfected, DCs failed to elicit antigen-specific TH17 cells.[120] CD40L treatment of human monocytes resulted in anti-mycobacterial activities.[119] By contrast, compared with the wild-type mice, CD40L-deficient mice remain resistant to *Mtb* infection, although these mice had fewer granulomas and fewer $CD4^+$ T cells in granulomas.[121] While these observations indicate that CD40 plays a significant role in anti-tubercular T cell-mediated host protection, some observations suggest otherwise leading to a paradox.

The paradox stems from the following findings. Firstly, the direct CD40 engagement on chronically *Mtb*-infected macrophages failed to elicit mycobactericidal activities[120] possibly because of complete subversion or switching to probacterial CD40 signalling. In fact, such observations were reported with *L. major* infection of BALB/c-derived macrophages.[122] Yet, whether similar possibilities

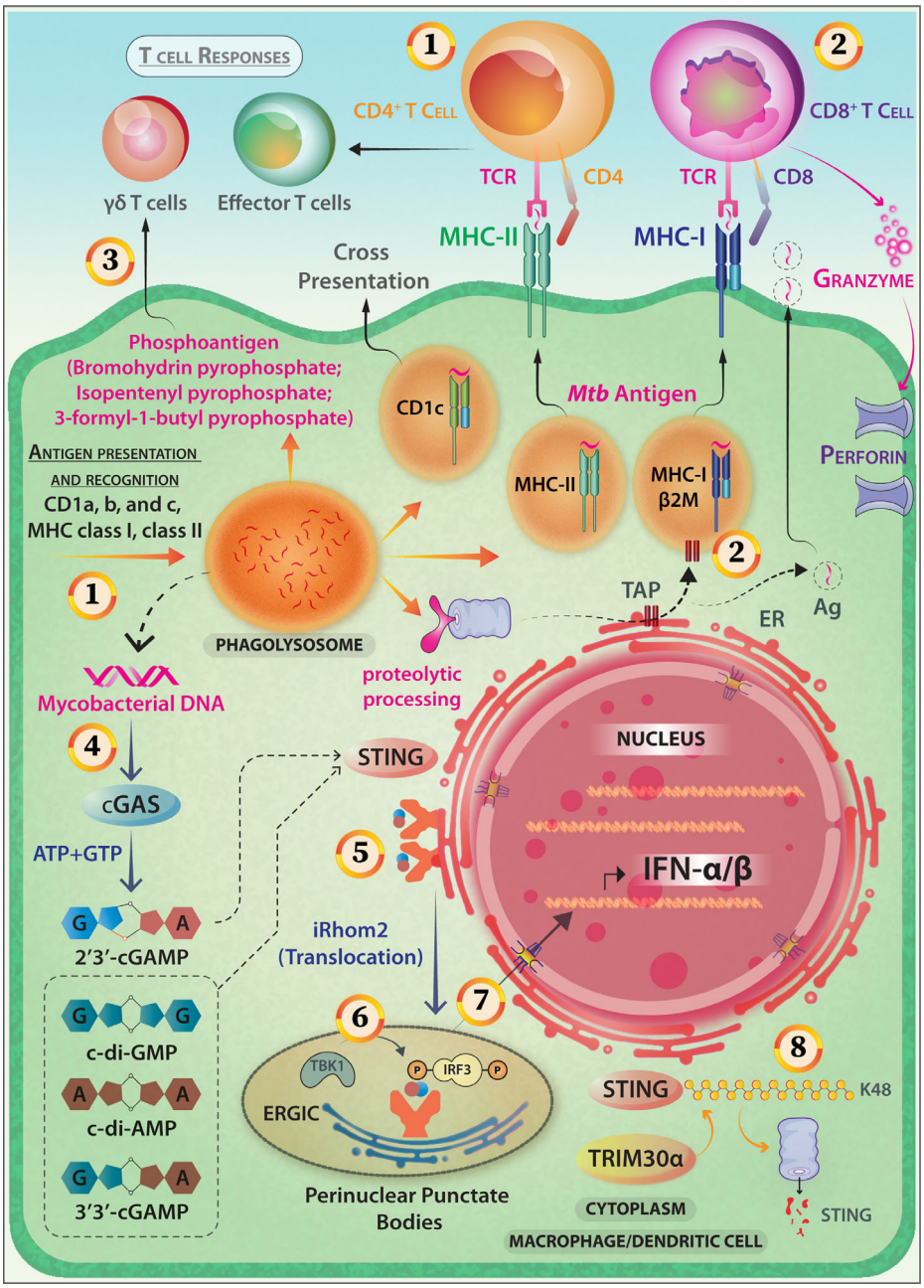

Figure 7. Antigen presentation, T-cell responses and activation of the cytosolic surveillance pathway (CSP) in the case of Mycobacterial infection of APCs. **(1)** Mycobacterial antigens can access both cytosolic and vacuolar antigen-processing pathways and are presented by the class-II MHC pathway inducing a potent CD4 response. **(2)** Presentation in the context of MHC-I (whereby $CD8^+$ T cell is activated) and CD1 (lipidic antigen) is also reported. **(3)** Novel phospholigands like bromohydrin pyrophosphate (BrHPP), Mycobacterial antigens (Isopentenyl Pyrophosphate, IPP and non-prenyl phosphoantigen 3-formyl-1-butyl-pyrophosphate) are potent elicitors of $V\gamma9V\delta2^+$ T cells. **(4)** *Mycobacterium* activates cytosolic sensor c-GAS, the STING/TBK1/IRF3 pathway through c-GAMP and induces Type I IFN-mediated innate immune responses. **(5)** Cyclic dinucleotides binding on STING induces its migration from the endoplasmic reticulum (ER) to form perinuclear punctate structures. This intracellular trafficking is mediated by iRhom2. **(6)** TBK-1 phosphorylates CTD-of STING and that results in IRF-3 recruitment and phosphorylation. **(7)** The IRF-3 homodimers translocate to the nucleus to activate the gene transcription of type-I IFNs. **(8)** TRIM30α, which is a negative-feedback regulator of STING via K48-linked polyubiquitination, marks it for proteasomal degradation. **(9)** Additionally, *Mycobacterium* actively employs SecA2 and ESX-1 secretion systems for releasing RNA into host cells and elicits IFN-β production through STING and IRF3 activation. Mycobacterial RNA activates the RIG-1-MAVS-TBK1-IRF-7 pathway (not shown).

exist in *Mtb* infection remains unexplored. Secondly, CD40-deficient mice succumbed to aerosolic low-dose *Mtb* infection because of deficient IL-12 production leading to impaired priming of IFN-γ-secreting T-cell responses but the CD40L-deficient mice remained resistant to the same infection.[121] These paradoxical results in CD40-deficient and CD40L-deficient mice implied the presence of an alternative ligand for CD40. Indeed, mycobacterial Hsp70 has been proposed to be an alternative ligand for CD40, as Hsp70 was coimmunoprecipitated with CD40 from *Mtb*-infected monocytic cell lines.[123] However, as Hsp70 is conserved from bacteria through humans, it remains to be seen whether mouse or human mono-mac cells expressed Hsp70 evokes intracellular signalling similar to that triggered by CD40L and elicits protection against the *Mtb* infection. Thirdly, CD40L-deficient mice developed anti-mycobacterial T-cell responses to the levels observed in the wild-type mice.

The data generated using the CD40-deficient or CD40L-deficient mice, or the mono-mac cells thereof, thus present a conundrum about the role of CD40 in *Mtb* infection. Recent mass-spectrometry based studies have identified nitric oxide-induced alterations in the expression of 1713 proteins in *Mtb*-infected macrophage-like cell line.[124] Nitric oxide can be generated *in situ* by the inducible nitric oxide synthetase, which can be induced by CD40 signalling.[125] It has also been shown that in response to such oxidative stresses, *Mtb* alters the phosphorylation of serine, threonine and tyrosine kinases.[126] It is possible that CD40-induced IL-10 exerts pro-mycobacterial effects, as reported for *Leishmania* infection in macrophages.[125] This would fit the conundrum, as CD40 was shown to induce IL-12 and IL-12-induced IFN-γ was shown to activate macrophages to trigger anti-mycobacterial effects such as by NO and reactive oxygen species productions.[127] Therefore, the same receptor CD40 signals in a contrasting manner when macrophages are chronically infected, or not, with *M. tuberculosis* and trigger counteractive effector functions.

CONCLUDING REMARKS

It is clear from the above account that *Mycobacterium* redirects or suppresses the immune response by intercepting the following processes: (1) the processing of the mycobacterial antigens by the antigen-presenting cells such as macrophages and DCs, (2) presentation of the processed *Mycobacteria*-derived antigens, (3) responsiveness of the T cells to the antigen-derived first signal and the ancillary signals from the costimulatory molecules and cytokines and (4) response of the *Mycobacterium*-infected macrophages to different cytokines. The conclusions from the analyses are expected to reveal the regulation of macrophage functions by CD40-CD40L interactions, negative regulators and dynamicity in the infection process. Such understanding will brace up novel aspects of macrophage–*Mycobacterium* interactions including the mechanisms of pathogenesis and possible immunotherapeutic targets.

ACKNOWLEDGMENT

The work had financial assistance from JC Bose National Fellowship (India) to BS [JCB/2018/000027].

AUTHOR CONTRIBUTIONS

Prashant chauhan: Resources; Software; Visualization; Writing-review & editing. **Jagneshwar Dandapat**: Supervision. **Arup Sarkar:** Supervision. **Bhaskar Saha:** Conceptualization; Formal analysis; Resources; Supervision; Writing-original draft; Writing-review & editing.

CONFLICT OF INTEREST

The authors declares no conflict of interest.

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