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REVIEW

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Trained immunity contributes to the prevention of *Mycobacterium tuberculosis* infection, a novel role of autophagy

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ABSTRACT

Mycobacterium tuberculosis (*M. tuberculosis*) is the pathogen which causes tuberculosis (TB), a significant human public health threat. Co-infection of *M. tuberculosis* and the human immunodeficiency virus (HIV), emergence of drug resistant *M. tuberculosis*, and failure to develop highly effective TB vaccines have limited control of the TB epidemic. Trained immunity is an enhanced innate immune response which functions independently of the adaptive/acquired immune system and responds non-specifically to reinfection with invading agents. Recently, several studies have found trained immunity has the capability to control and eliminate *M. tuberculosis* infection. Over the past decades, however, the consensus was adaptive immunity is the only protective mechanism by which hosts inhibit *M. tuberculosis* growth. Furthermore, autophagy plays an essential role in the development of trained immunity. Further investigation of trained immunity, *M. tuberculosis* infection, and the role of autophagy in this process provide new possibilities for vaccine development. In this review, we present the general characteristics of trained immunity and autophagy. We additionally summarize several examples where initiation of trained immunity contributes to the prevention of *M. tuberculosis* infection and propose future directions for research in this area.

Abbreviations: ARE: antioxidant response element; ATG: autophagy related; BCG: Bacillus Calmette–Guérin; DCs: dendritic cells; ER: endoplasmic reticulum; ESAT-6: early secretion antigenic target-6; ESX-1; ESAT-6 secretion system-1; HAT: histone acetyltransferase; HBV: hepatitis B virus; HDAC: Histone deacetylase; HIV: human immunodeficiency virus; HO-1: haem oxygenase 1; HSC: haematopoietic stem cell; HSPCs: haematopoietic stem and progenitor cells; IFN-γ: interferon γ; IL: interleukin; ILCs: innate lymphoid cells; Keap1: Kelch-like ECH-associated protein 1; LC3: microtubule-associated protein 1-light chain 3; LPS: lipopolysaccharide; LTBI: latent tuberculosis infection; *M. tuberculosis*: mycobacterium tuberculosis; NADPH oxidase 2; NFAT: nuclear factor of activated T cells; NO: nitric oxide; NOX2: NQO1: NADPH quinone oxidoreductase; Nrf2: nuclear factor erythroid 2-related factor-2; p62: sequestosome 1; ROS: reactive oxygen species; SOD: superoxide dismutase; TB: tuberculosis; TF: transcription factor; TLR4: Toll-like receptor 4; TNF-α: tumour necrosis factor; Ub: ubiquitin; ULK1; unc-51-like kinase 1; WD: western diet

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Introduction

Tuberculosis (TB) is one of the leading causes of mortality worldwide. In 2018, there were approximately 10 million people infected with *Mycobacterium tuberculosis* (M. *tuberculosis*) globally, and 8.6% of the TB patients were also infected with human immunodeficiency virus (HIV) [1]. Although the lung is the most commonly affected organ, M. *tuberculosis* can spread via blood vessels to cause infection in multiple organ systems [1]. For years, studies have focused extensively on the role of adaptive immune cells, such as T and B cells, in anti-M. *tuberculosis* infection [2], however the contribution of innate immune cells regarding this aspect has not been investigated in depth. Recently, studies have shown that innate immunity plays an essential role in controlling M. *tuberculosis* infection [3,4]. These findings indicate prior to development of adaptive immunity, innate immunity is adequate in controlling M. *tuberculosis* infection which may explain why some individuals are not infected even after extensive exposure to *M. tuberculosis* [3–5]. Early clearance of M. *tuberculosis* is closely associated with enhanced non-specific innate immune responses, such as mechanisms during induction of trained immunity [6]. Enhanced heterologous innate immunity may be a form of trained

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immunity, first termed by Netea [7] in 2011. Epigenetic and metabolic reprogramming are central mechanisms for the development of trained immunity against M. *tuberculosis* infection [8]. Interestingly, autophagy could control trained immunity induced by BCG via manipulation of histone modifications [9]. BCG-induced trained immunity is cancelled while inhibiting autophagy in monocytes from healthy human volunteers, suggesting autophagy is a central event in induction of trained immunity [9]. Moreover, single nucleotide polymorphisms (SNPs) in autophagy-associated genes such as ATG2B control trained immunity, as well as the heterologous therapeutic effects of BCG in bladder cancer patients [9]. Whether this process contributes to containment of M. *tuberculosis* infection remains to be elucidated. Increasing evidence has shown trained immunity contributes to clearing pathogens, including M. *tuberculosis* [8]. An increasing number of studies have provided evidence showing trained immunity can promote the development of certain inflammatory diseases [10]. Improved understanding of the mechanism of trained immunity during M. *tuberculosis* infection can provide opportunities for TB treatment.

Trained immunity and its induction (BCG, β-glucan, LPS and any others)

The immune system is categorized into adaptive/acquired immunity (specific) and innate immunity (non-specific). Adaptive immunity can generate pathogen-specific T-cell receptors (TCRs) and B-cell receptors (BCRs, also known as antibodies), which enables immunological memory cells to remember previous encounters. A highly specific immunological response is promptly initiated upon a subsequent encounter with the same pathogen. Compared to adaptive immunity, innate immunity is generally viewed as primitive and less sophisticated, lacking the ability to distinguish the type of pathogen or the number of contacts. In 1961, Ross et al. [11] found that defence ability of tobacco after being infected by the Tobacco mosaic virus (TMV) was substantially potentiated. Similar phenomena were observed in various invertebrates and animals [12]. Furthermore, Kleinnijenhuis et al. [13] suggested that production of interferon-gamma (IFN-γ) in healthy volunteers vaccinated with Bacillus Calmette–Guérin (BCG) increased 4- to 7-fold and led to 2-fold production of monocyte-derived cytokines, such as tumour necrosis factor (TNF) and interleukin 1β (IL-1β), in response to unrelated pathogens. In addition to T and B cells, innate immune cells are able to mount a de facto immunological memory similar to that observed in acquired immunity and corresponding to the defence mechanisms of lower organisms, thereby increasing their resistance to secondary infections. This is suggestive of an ancient biological process. The capability of innate immune cells is more sophisticated than initially thought. The phenomenon of innate immune memory is termed trained immunity [7]. Unlike the precise response of adaptive immunity, trained immunity is non-specific. Innate immune cells involved in this process include monocytes/macrophages, NK cells, neutrophils, dendritic cells (DCs), and innate lymphoid cells (ILCs) [14]. There is increasing evidence that monocytes/macrophages and NK cells can exhibit inherent immune memory characteristics [8]. This phenomenon supplements and even rebuilds our understanding of the function of the natural immune system.

The trained immunity state is orchestrated by epigenetic reprogramming involving histone tail modifications and reconfiguration of chromatin, resulting in gene expression rewiring [8]. Modifications are mainly methylation, acetylation, or phosphorylation. Methylation of histone is related to the condensation of chromatin, which affects binding of transcription factors (TF) to specific DNA motif which leads to gene silencing. On the other hand, acetylation is linked to activation of gene transcription. Positioning of methylated amino acid residues and the number of bound methyl groups at least partially determine the final effect of epigenetic reprogramming [15]. The enzymes that catalyse methylation [Lysine methyltransferase (KMT)] and demethylation [Lysine demethylase (KDM)] of histones demonstrate significant specificity toward amino acid positions within the histone tail [16]. Activation of gene transcription is a result of histone 3, lysine 4 (H3K4), H3K36, and H3H79 methylation, while inhibition of gene transcription is typically associated with methylation of H3K9, H3K27, and H4K20 [15]. Acetylation is a reversible histone modification catalysed by histone acetyltransferase (HAT), which can transfer acetyl groups from acetyl coenzyme A (AcCoA) to lysine residues and is related to activation of gene transcription. Histone deacetylase (HDAC) can reverse the above effects of HAT. Balance between HAT and HDAC activity is an important determinant of gene regulation and has become a potential therapeutic target in treatment of various diseases. Immunometabolism changes are also observed in trained innate cells. Although it has long been recognized that changes in oxygen and glucose consumption can adapt to the metabolic requirements of activated macrophages, the importance of intracellular metabolism in shaping the immune response has only recently been discovered [17]. Studies have indicated Akt / mTOR / HIF1α-mediated aerobic glycolysis is the metabolic basis of the trained immunity [18]. Other metabolic pathways such as glycolysis, TCA cycle, cholesterol synthesis, glutaminolysis, and accumulation of

fumaric acid are involved in the development of monocyte-trained immunity [19].

Many stimuli, such as bacteria, fungi, parasites, and viruses, as well as their components, can induce trained immunity (Figure 1). For example, *Candida albicans* (an opportunistic pathogen in humans), which inhabits the mouth, gastrointestinal tract, and vagina. Human monocytes stimulated by β-glucan, a part of the cell wall of *Candida albicans*, develop immunity against fungi as well as heterologous infections [8]. Similar to (1,3) / (1,6) -β-glucan derived from fungi, Pan et al. [20] found that monocytes/macrophages from mouse bone myeloid and human THP-1 cells primed with β-glucan from dietary fibre oats *in vitro*, structured as (1,3)/(1,4)-β glucan, enhanced TNF-α and IL-6 mRNA expression and production in response to re-stimulation of TLR-2/4 ligands. These findings indicate oat-derived β -glucan can also induce trained immunity. Like β-glucan, chitin is a significant component of the fungal cell wall; therefore, it may also affect the host immunity state. Rizzetto et al. [21] demonstrated that chitin-stimulated human monocytes via *Saccharomyces cerevisiae* have potentiated the ability to eliminate microbes (e.g. *Candida albicans*, *Staphylococcus aureus*, or *Escherichia coli*) compared to unstimulated monocytes. The differing nature of primary stimuli can induce opposite immune states in the host: immune tolerance and trained immunity. Lipopolysaccharide (LPS), also known as endotoxin, is the primary component of the cell wall of most Gram-negative bacteria [22]. Varying doses of LPS have different effects on the inflammatory response. Previous studies reported that initial contact of monocytes/macrophages with high doses of LPS induce a tolerance state to avoid excessive immune response which may result in tissue damage during secondary stimulation, whereas low concentrations of LPS skew the cells toward a trained immunity state to eliminate invading pathogens [23]. This phenomenon is a result of differing gene expression patterns [24]. The IRG1-Itaconate-SDH axis plays a vital role in development of immune tolerance and trained immunity [25]. A high-dose LPS-derived tolerance state may be reversible by β-glucan through inhibiting expression of IRG1, which could also restore capability of human macrophages to produce cytokines [25]. β-glucan-induced trained immunity may have the potential to reverse immunoparalysis. Studies utilising *Anopheles gambiae* mosquitoes have shown initial *Plasmodium falciparum* infections lead to an innate immune memory, thereby partially contributing to prevention of secondary infections with Plasmodia [26]. This indicates the causative pathogen of malaria, *Plasmodium falciparum,* is also an inducer of trained immunity [26]. Besides Plasmodia, a trained immunity state could also be triggered by certain viruses, such as the hepatitis B virus (HBV), a causative agent of liver inflammation and cancer. Hong et al. [27] demonstrated exposure of neonates to HBV in utero induces a trained immunity state, which intensifies the ability of immune cells to respond to bacterial infections *in vitro*. Compared with healthy neonate controls, mononuclear cells from cord blood of neonates delivered by HBV+ mothers produced more IL-12p40 and IFN-α2 and less IL-10 against unrelated bacterial pathogens [27]. One of the most studied agents in the aspect of trained

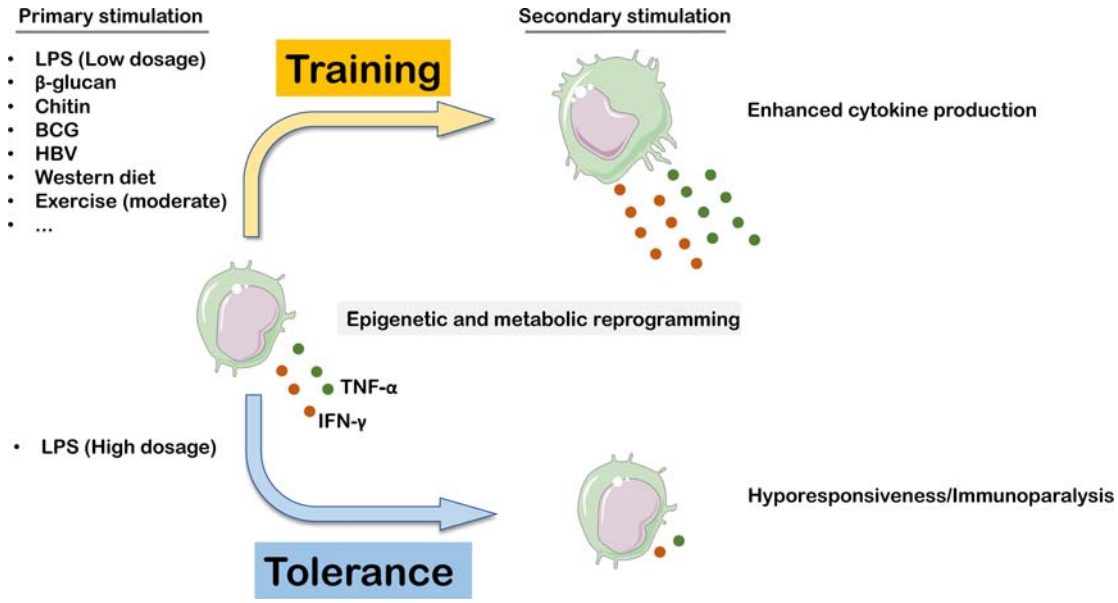

Figure 1. Innate immune memory induced by varying stimuli. Trained immunity state can be induced by diverse agents, which enhances the immunological response of innate immune cells to either the same or unrelated secondary stimulation. Conversely, a high dosage of LPS drives innate immune cells into a tolerance state with immunological hyporesponsiveness to reinfection. Similarly, both training and tolerance states are orchestrated by epigenetic and metabolic reprogramming. Elements of some figures were made using Servier Medical Art, (https://smart.servier.com).

immunity is the BCG vaccine. Mice vaccinated with BCG can mount protective effects against other bacteria, such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium*, or *Schistosoma mansoni*. This heterologous immunity was also observed in mice lacking T and B lymphocytes [13,28]. BCG was discovered in the 1940s and protects against tuberculosis and other infections, which has significantly improved the survival rate of infants [29–31]. Recently, it was noted that trained immunity induced by BCG vaccination could reduce susceptibility to and the severity of SARS-CoV-2 infection, which is the virus known to cause the recent COVID-19 pandemic [32].

A trained immunity state can also be induced by exercise and diet. Studies involving race horses suggested moderate exercise led to altered mRNA expression of certain TLRs and cytokines produced by monocytes after *in vitro* treatment with TLR ligands [33]. Contrarily, systemic inflammation induced by a Western diet (WD) in *Ldlr−/−* mice led to increased proliferation of myeloid progenitor cells and enhanced innate immune responses, suggesting a WD could induce an enhanced immunological state; however, NLRP3-mediated trained immunity has possible detrimental effects in inflammatory diseases [34].

Trained immunity induced by different stimuli and its role in *M. tuberculosis* infection

Two separate events are needed for one to develop TB: (1) infection with *M. tuberculosis* and (2) progression of the disease to active tuberculosis, especially in immunocompromised individuals. There has recently been more focus on exploring why some individuals do not become infected with *M. tuberculosis* during an initial encounter. For years, however, the scientific community has focused on why some individuals develop active TB while others do not [5]. Strong epidemiological evidence showed some individuals develop neither latent tuberculosis infection (LTBI) nor active tuberculosis, even after heavy exposure to *M. tuberculosis* in closed environments [35]. In 1966, there was an outbreak of tuberculosis in an enclosed naval ship compartment. Of the 66 members enlisted at risk, 47 had converted tuberculins and six had active disease, whereas the remaining 13 crew members were negative for TST during the initial study and remained so at follow-up after six months [35]. These exposed individuals almost certainly inhaled *M. tuberculosis*, but the infection was likely controlled or cleared through innate immune protection mechanisms. This innate immune protection functions independently of adaptive immunity and is termed early clearance [36]. Although the molecular mechanisms underlying early clearance remain largely unknown, a trained immunity state at least partially results in the elimination of *M. tuberculosis*. Before the development of adaptive immunity, innate immunity has the ability to contain or clear *M. tuberculosis* infection [3,5].

BCG-induced trained immunity in *M. tuberculosis* infection

BCG is currently the only licenced vaccine to protect against *M. tuberculosis* infection and has been used to vaccinate >90% of new-born infants annually [37]. In clinical trials, BCG vaccination has been shown to provide 0-80% protection in adults [38]. The reasons for this inconsistency are not fully understood. As discussed above, some individuals do not develop LTBI or active TB when exposed to patients infected with *M. tuberculosis*. Early clearance plays a key role in this process and is closely associated with a positive history of BCG vaccination. A BCG-induced trained immunity state may be responsible for early clearance of *M. tuberculosis* [5,6]. Several studies have demonstrated trained immunity induced by BCG at least partially led to resistance to *M. tuberculosis* infection. For instance, in an ex vivo study, enhanced anti-mycobacterial activity in *M. tuberculosis*-infected macrophages derived from monocytes was observed in BCG-vaccinated individuals (identified as responders). Promoters with a differential DNA methylation pattern were enriched in response to BCG among genes belonging to immune pathways in responders, indicating BCG-induced trained immunity contributes to containment of *M. tuberculosis* replication [39]. Monocytes trained by BCG resulted in increased reactive oxygen species (ROS) expression, which kills *M. tuberculosis* by destroying the redox homeostasis of the pathogen, while training with β-glucan led to downregulation of ROS production [28]. These monocytes expressed CD11 and TLR4 which are crucial in priming phagocytosis, indicating improved ability to engulf *M. tuberculosis* by trained monocytes [13,40]. This enhanced function of trained monocytes induced by BCG persisted for at least three months despite their short life span in circulation [13]. Based on this puzzling finding, Kaufmann et al. [41] found that administration of BCG can access the bone marrow and trigger expansion of haematopoietic stem cells (HSC) and enhance myelopoiesis. HSC reprogramming was sustained regardless of persistent exposure to BCG, which may contribute to long-term effects of trained monocytes, indicating targeting HSC provides a novel strategy for vaccine development. Of note, the training effects of BCG vaccination are NOD2 and Rip2 dependent and NOD2 is a participant in initiation of autophagy [13]. Induction of autophagy suppressed

intracellular survival of *M. tuberculosis* and maintained homeostasis [42]. Inhibition of autophagy by 3-methyladenine (3MA) eradicates the BCG-induced trained immunity state by blocking epigenetic reprogramming of monocytes at the level of H3K4me3, suggesting a pivotal role of autophagy in development of trained immunity [9].

The BCG vaccine also has the ability to induce a trained immunity state of NK cells. Like monocytes, trained NK cells were also accompanied by epigenetic modifications at the level of H3K4me3. A study conducted by Kleinnijenhuis et al. [43] suggested that NK cells isolated from BCG-vaccinated volunteers enhanced production of proinflammatory in response to both mycobacteria and unrelated pathogens. Moreover, NK cells were partially responsible for prolonged survival in SCID mice following BCG vaccination in a murine model treated with a lethal dose of *Candida albicans* [43]. Besides BCG, *M. tuberculosis* is capable of inducing trained immunity. Several human studies have shown robust control of BCG outgrowth was observed only in individuals with recent exposure to *M. tuberculosis*. This phenomenon was correlated with expression of CXCR3 ligands (CXCL9, CXCL10 and CXCL11), which represents new markers of trained immunity. These data suggest that *M. tuberculosis* itself can induce trained immunity. Whether this contributes to the prevention of mycobacteria infection requires further investigation [44]. Trained immunity induced by BCG vaccination conferred enhanced response of the innate immune system not only against *M. tuberculosis* but also other unrelated infections, which could explain why BCG vaccination reduced all-cause morbidity and mortality in infants [29–31]. After approximately a century of BCG vaccination, we still do not fully understand the basis for BCG protection. An improved understanding of the precise immunological pathways is crucial in control of *M. tuberculosis* infection.

β-glucan induced trained immunity in *M. tuberculosis* infection

β-glucan, a polysaccharide component of fungal cell wall, is one of the most widely studied trained immunity stimulants. Early studies have shown that macrophage activation via dectin-1, a C-type transmembrane lectin receptor, induces specific epigenetic hallmarks which lead to trained immunity [18]. Several studies suggest a β-glucan-induced trained immunity state contributes to protection against *M. tuberculosis* infection. For example, β-glucan treatment during mycobacterium infection led to an elevated level of intracellular ROS and decreased survival of M. bovis BCG in human macrophages mediated by the dectin-1 pathway. This phenomenon was not observed in human macrophages with *M. tuberculosis* infection, indicating differing pathogenic properties in M. bovis BCG versus virulent *M. tuberculosis* [45]. Nevertheless, a unique mechanism by which *M. tuberculosis* survives within macrophages needs to be elucidated. Two similar findings have been reported by Hetland et al. [46,47] suggesting β-glucan conferred a protective effect against M. bovis BCG and *M. tuberculosis* infection in BALB/c mice and macrophage cultures, respectively. Macrophages can inhibit *M. tuberculosis* survival through activation of STAT-1 and NF-κB pathway which lead to nitric oxide (NO) production following β-glucan training both *in vivo* and *ex vivo*. Stimulation of dectin-1 signalling with β-glucan also triggers calcium influx, activating nuclear factor of activated T cells (NFAT) signalling. NFAT signalling then facilitates accessibility of DNA to transcription machinery and consequently enhances gene transcription in secondary stimulation of the cells [48]. Gupta et al. [49] found that use of the RyR2 (a receptor for regulating intracellular calcium) agonist caffeine increased calcium influx and was capable of killing intracellular *M. tuberculosis* via complete maturation of phagosomes in THP-1 macrophages [50]. β-glucan possibly impedes *M. tuberculosis* growth by increasing calcium influx mediated by phagocytosis. Like the effects of the BCG vaccine, recent studies have shown trained immunity induced by β-glucan acts at the level of haematopoietic stem and progenitor cells (HSPCs), preserving their capacity to yield myeloid-skewed immune cells 12 weeks post-transfer into untrained recipients. This promotes a beneficial response in re-stimulation [51]. Whether this process confers the ability to reduce mycobacterial loads remains elusive.

LPS-induced trained immunity in *M. tuberculosis* infection

Conceptually, both tolerance state and enhanced response state (trained immunity) belong to innate immune memory. The final effects of tolerance or trained immunity are closely correlated with the strength of the external stimulus which is the primary contact. One of the most widely studied components in this aspect is LPS, which can programme innate immune cells into different immune states with varying expression of pro- and anti-inflammatory mediators according to differing concentrations of LPS [23]. As mentioned previously, very low doses of LPS (picograms/mL range) induce a trained immunity state, whereas tolerance state is resultant of high concentrations of LPS (nanograms/mL range), and mechanistically causes GSK3 and Akt activation, respectively [23]. LPS-induced tolerance is a hyporesponsive state of macrophages due to receptor desensitization [52]. In a study of LPS tolerance in mice macrophages, Froster et al. [53] illustrated gene-

specific chromatin modifications were associated with gene silencing (*tolerizeable*) to prevent excessive inflammation and priming of other genes (*non-tolerizeable*) encoding antimicrobial molecules to protect against foreign infection. At the molecular level, LPS is recognized by TLR4. Early studies have shown LPS activates macrophages via TLR4, which plays a pivotal role in the inflammatory response to infection via improving production of ROS and promoting a protective immune response [54]. Increased expression of TLR4 and NADPH oxidase (mainly NOX2) leads to enhanced capacity of phagocytosis and ROS production, resulting in elimination of *M. tuberculosis* (Figure 2). This finding was observed in PMA-primed THP-1 macrophages upon LPS treatment [40]. The LPS-induced protective effects against *M. tuberculosis* infection are dependent on the TLR4-NOX2 axis; inhibition of TLR4 and/or NOX2 disrupted these bactericidal activities [40]. A tolerance state should be induced since LPS (100 ng/mL) was used in this study and a protective effect was observed. Whether this process contributing to prevention of *M. tuberculosis* infection is associated with gene-specific control of inflammation during LPS tolerance remains to be elucidated. Moreover, a similar study conducted by Fang et al. [55] demonstrated that restoration of autophagy, which is an important protective mechanism against *M. tuberculosis* infection, was screened in LPS training of THP-1 derived macrophages (Figure 2). We conclude that both LPS-induced trained immunity and LPS tolerance at least partially contribute to elimination of *M. tuberculosis* infection. Despite these advancements in our understanding, underlying epigenetic mechanisms remain largely unclear.

The role of autophagy in trained immunity against *M. tuberculosis* infection

Macroautophagy (hereafter referred to simply as autophagy) is a potent effector mechanism, which is vital in maintaining cellular homeostasis through sequestering damaged organelles, long-lived proteins, bacterial products and other cytoplasmic components into a double-membrane vesicle (the autophagosome) and subsequently delivering these products to degradation in autolysosome [56]. The function of autophagy is not limited to the simple elimination of cytosolic materials, but also serves as a dynamic recycling system which provides new substrates for energy metabolism and de novo protein synthesis. These processes are important for cellular quality control [56].

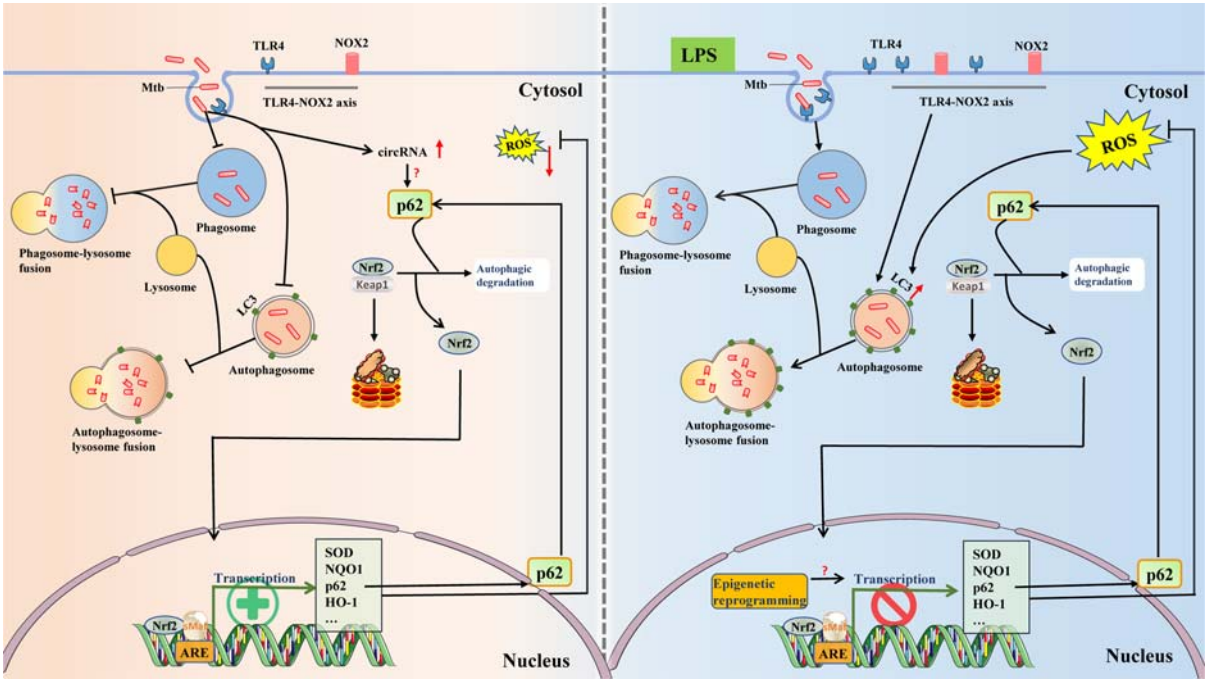

Figure 2. LPS-induced trained immunity state contributes to against *Mycobacterium tuberculosis* (*M. tuberculosis*) infection in macrophages. Following uptake by macrophages, *M. tuberculosis* can inhibit phagosome maturation and fusion of the phagosome with the lysosome, as well as expression levels of LC3 on the autophagosome membrane. It has been reported that *M. tuberculosis* can escape from the phagosome and reside in the cytosol via perforations in the phagosome membrane (data not shown). These combined efforts lead to a niche for *M. tuberculosis* persistence within macrophages. Activation of p62 via competitively binding of *M. tuberculosis* to Nrf2 with Keap1, resulting in translocation of Nrf2 into the nucleus, followed by incorporation with sMaf proteins to induce anti-oxidative cytokine expression. Interestingly, these antioxidant proteins lead to the elimination of ROS, which has an important bactericidal activity on invasive pathogens, including *M. tuberculosis* (left). Recent studies have found that restoration of bactericidal activities (phagocytosis, autophagy, and ROS expression) was screened in macrophages when challenged with LPS. Besides, LPS treatment can enhance the TLR4 and NOX2 expression levels. All of these effects are dependent on the TLR4-NOX2 axis (right). Elements of some figures were made using Servier Medical Art, (https://smart.servier.com).

Many stimuli, even environmental stresses such as starvation, oxidative stress, rapamycin, and certain cytokines, can all induce autophagy [57]. Once autophagy is induced, two primary steps are required for the formation of the autolysosome: (1) an isolation membrane (also known as the phagophore) envelops a portion of cytoplasm and forms double-membrane vesicles, namely autophagosomes, and subsequently (2) fuse with lysosomes, forming autolysosomes by which the cell degrades the contents of autophagosomes. Formation of the autolysosome recruits a variety of key proteins at the differential stage. For instance, the unc-51-like kinase 1 (ULK1) complex, comprising the ULK1, autophagy-related (ATG) proteins (e.g. ATG13, ATG101) and the focal adhesion kinase family-interacting protein of 200 kDa (FIP200), ATG9A, and phosphatidylinositol 3-kinase (PI3K) complex are needed at the initial stage of autophagy as inhibition of these autophagy factors leads to failure of phagophore formation and no accumulation of autophagy substrates [58]. In a period of isolation membrane formation and elongation, the WD-40 repeat domains, phosphoinositide-interacting 2 (WIPI2) and ATG2A/B, are recruited [58]. Closed autophagosome could be visualized using a maker, syntaxin 17 (STX17), as it is recruited immediately before or after the completion of the autophagosome [59]. STX17 exists in the endoplasmic reticulum (ER) and mitochondria, inferring not all STX17 represents a closed autophagosome. At the end of this process, the complete autophagosome fuses with the lysosome, forming the autolysosome which degrades contents within.

General autophagy was originally viewed as a simple, non-selective catabolic process that consumes dysfunctional and unnecessary intracellular components to generate new blocks for cell and tissue renovation; however, accumulating evidence has proven that autophagy contributes to the restriction and killing of intracellular invading pathogens including *M. tuberculosis*, though knowledge of the underlying molecular mechanisms is far from complete [60]. *M. tuberculosis* has evolved several elaborate strategies to arrest phagosome maturation upon its phagocytosis, including inhibition of fusion between phagosome and lysosome and perforation of the phagosome, resulting in translocation of the organism from the phagosome to the cytosol [61]. The ESAT-6 secretion system-1 (ESX-1) is a major virulence factor which is encoded by the region of difference-1 (RD1) gene. ESX-1 has been identified as a method for *M. tuberculosis* to impair the autophagic flux, block autophagosome maturation, and escape the phagosome in human DCs and macrophages [61,62]. In addition to the ESX-1 system, *M. tuberculosis* may access the cytosol through an additional approach which has haemolytic activity like ESX-1 [63]. Notably, the vaccine strain BCG and avirulent (H37Ra) strain lack the virulence factor ESX-1, which may explain why BCG and H37Ra is attenuated as the restoration of the capacity to inhibit autophagy was observed in those recombinant strains in which ESAT-6 secretion was re-established by genetic complementation [62]. Based on this finding, drugs that inhibit ESX-1 may potentially attenuate *M. tuberculosis* infection. Studies found the early secretion antigenic target-6 (ESAT6) of ESX-1 could drive macrophages into M2 polarization [64]. Expression of ESAT6 is responsible for increased *M. tuberculosis* survival through modulating miR-30a production and has been identified as a phenotype favouring *M. tuberculosis* residence in macrophages [64].

Escaping into the cytosol does not mean *M. tuberculosis* can survive continuously; conversely, it activates autophagy, which can override the inhibition caused by *M. tuberculosis* pathogen subverting the phagosome-lysosome fusion, and subsequently contributes to the elimination of bacteria (Figure 3) [57,65]. Previously, some studies demonstrated that activating autophagy via IFN-γ and other agents has the capacity to facilitate phagolysosome formation and suppress *M. tuberculosis* survival in macrophages. These findings suggest induction of autophagy is related to a protective effect against invading microbes [42,66]. In line with these findings, Ponpuak et al. [67] found that autophagic protection against *M. tuberculosis* is through the lytic and antimicrobial properties of the autolysosome which are stronger than that of a conventional phagosome. However, *M. tuberculosis* pathogen could evade and manipulate autophagy by the virtue of several developed counter-strategies which have created a niche for its survival (Figure 3). First, the virulent *M. tuberculosis* H37Rv can upregulate the production of IL-6 which selectively attenuates IFN-γ-induced autophagosome biogenesis by inhibiting the ATG12-ATG5 complex [66]. Second, *M. tuberculosis* potentiates the expression of 42-kDa protein which is the product of a unique protein-coding gene, the enhanced intracellular survival gene (*Eis*), which has been known to directly increase loads of *M. tuberculosis* through manipulating macrophage autophagy [68–70]. Silencing the *Eis* gene significantly enhances autophagic vesicles and autophagosome formation [70]. A recent investigation identified LprE, a lipoprotein of *M. tuberculosis*, as a virulence factor which subverts host immune response as a result of suppression of autophagy [71]. Third, recent studies found that *M. tuberculosis* can harness certain microRNAs (miRNAs) which subvert TLR signalling and autophagy to support self-survival, e.g. miR-27a, miR-129-3p, miR-33. Inhibition of miRNAs impeded *M. tuberculosis* survival in cells [72]. Fourth, *M. tuberculosis* can arrest RAB7-dependent bacterial autophagosome maturation into an autolysosome,

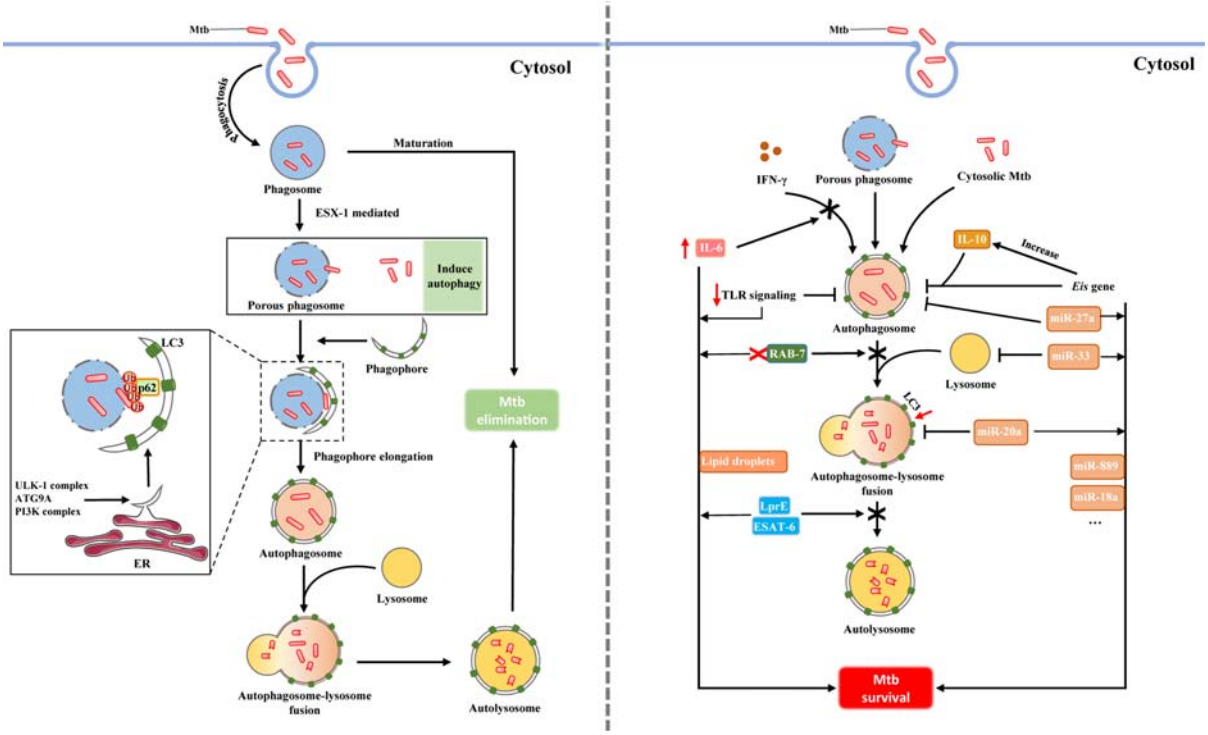

Figure 3. *Mycobacterium tuberculosis* (*M. tuberculosis*) has evolved several counterstrategies to evade autophagic elimination. Generally, *M. tuberculosis* can be sequestered and delivered for degradation by the phagosome upon phagocytosis by macrophages. However, *M. tuberculosis* can escape the phagosome and access to the cytosol via ESX-1, which subsequently activates the autophagic process, another protective degradative pathway. Cytosolic localization of *M. tuberculosis* with tagged ubiquitin is then delivered to phagophore formation site, ER. Further, the autophagosome fuses with the lysosome forming the autolysosome which degrades contents within. The combined effect of these events contributes to *M. tuberculosis* elimination (left). Unexpectedly, *M. tuberculosis* has evolved several countermeasures that create a niche for its survival to evade and even manipulate host autophagic processes. *M. tuberculosis* can directly upregulate IL-6 expression to selectively inhibit IFN-γ-induced autophagy. *M. tuberculosis* possesses a unique gene, *Eis*, by which mycobacteria arrests autophagosome formation and increase IL-10 production to block autophagy. Notably, *M. tuberculosis* evades autophagic elimination by using certain miRNAs (e.g. miR-20a, miR-27a, miR-33) in inhibiting autophagy. We also displayed other factors favouring mycobacterial survival in macrophages (right).

leading to inhibition of mycobacterial killing [73]. Apart from manipulation of autophagy, several novel mechanisms adopted by *M. tuberculosis*, such as membrane trafficking and integrity and cell death protect it from intrinsic immune bactericidal system, leading to persistent infection [69]. Autophagy, nevertheless, is a protective immune response which in part contributes to the intracellular fight against *M. tuberculosis* infection. We still do not fully understand the molecular mechanisms through which cells clear the intracellular insults, including *M. tuberculosis*. This impairs our ability to utilise the therapeutic potential of autophagy.

As previously mentioned, autophagy is a key player in manipulating epigenetic reprogramming in the development of trained immunity induced by BCG [9]. Failure of autophagy inhibits the BCG- or β-glucan-induced trained immunity *in vitro* [9]. In a recent study, *M. tuberculosis* infection was found to attenuate autophagy. LPS, however, could restore function of autophagy inhibited by *M. tuberculosis*, which is also an inducer of trained immunity [55]. It is important to determine if induction of trained immunity enhances autophagic function in this study or conversely, LPS-induced autophagy facilitates the biogenesis of a trained immunity state. Given that autophagy involves the development of trained immunity, it is critical to determine the exact mechanism by which autophagy influences epigenetic rewiring upon training. Further studies are needed to establish the connection between autophagy and trained immunity in protection against TB.

Conclusions and prospect of trained immunity as a target of anti-*M. tuberculosis* infection

TB is a communicable disease, which is spread by *M. tuberculosis* containing aerosol droplets expelled into the air by infected individuals. Failure to develop a highly effective anti-*M. tuberculosis* vaccine has limited control of the TB epidemic. The adaptive immune system generates a repertoire of *M. tuberculosis*-specific T cells or mediates via IFN-γ, and has been considered the chief immune mechanism in controlling *M. tuberculosis* infection and the foundation for effective vaccination [74]. Despite its relevance, the

acquired immune system has been viewed as the only protective mechanism during *M. tuberculosis* infection [75]. Vaccine strategies have been built upon the narrow definition of immunological memory, aiming to exploit cell-mediated immunity [8]. On the contrary, Comas et al. [76] found that *M. tuberculosis* may benefit from recognition of T cells with conserved epitopes, thereby benefiting their survival. In a human trial, the MVA85A candidate vaccine targeting T-cell function mediated by IFN-γ failed to achieve expected protection [77]. The emergence of the concept of trained immunity has challenged the dogma that only acquired immune cells mount immunological memory and has shocked the foundation of modern vaccinology. Of note, evidence has shown that trained immunity state has the ability to contain and eliminate pathogens, including *M. tuberculosis*. It is plausible that trained immunity may play an important role in immunocompromised patients, such as those co-infected with *M. tuberculosis* and HIV, which remains the leading comorbidity and poses a great challenge for global TB control. Hence, induction strategies should also attempt to provide a trained immunity state, which gives the host a pool of primed inherent cells with enhanced capabilities to eliminate pathogens and are ready to act before an initial encounter with *M. tuberculosis*. Of note, Bickett and colleagues have demonstrated BCG-induced protection against *M. tuberculosis* infection prior to induction of adaptive immunity and in the absence of NOD2 required for trained immunity, highlighting the importance of innate immune cells in reducing the burden of *M. tuberculosis* [78].

A trained immunity state was originally viewed as a beneficial effect as it confers broad immunological protection. Nevertheless, trained immunity is not always amicable. For instance, silica exposure induces lung damage, extracellular self-DNA release and STING activation, which impair host control of *M. tuberculosis* infection via initiation of type 2 immunity [79]. Furthermore, although increased secretion of pro-inflammatory cytokines partially contributes to containment and elimination of pathogenic insults, hyperinflammation caused by trained immunity might result in the development or maintenance of several inflammatory diseases [10]. A growing body of evidence suggests epigenetic reprogramming resulting in functional and transcriptional alternation in innate immune cells, may promote pathogenesis of multiple diseases including atherosclerosis, diabetes mellitus, chronic inflammatory disorders and neurodegenerative disorders, as it was reviewed by Włodarczyk et al. [10]. Therefore, inhibiting or reversing the changes caused by trained immunity might be a promising therapeutic target in these disorders. Taking all current evidence into account, it is important to illustrate the mechanisms underlying trained immunity and utilize other aspects of the immune system besides adaptive immunity in the development of vaccines.

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