

Trained immunity: a new avenue for tuberculosis vaccine development

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Adaptive immunity towards tuberculosis (TB) has been extensively studied for many years. In addition, in recent years the profound contribution of innate immunity to host defence against this disease has become evident. The discovery of pattern recognition receptors, which allow innate immunity to tailor its response to different infectious agents, has challenged the view that this arm

of immunity is nonspecific. Evidence is now accumulating that innate immunity can remember a previous exposure to a microorganism and respond differently during a second exposure. Although the specificity and memory of innate immunity cannot compete with the highly sophisticated adaptive immune response, its contribution to host defence against infection and to vaccine-induced immunity should not be underestimated and needs to be explored. Here, we present the concept of trained immunity and discuss how this may contribute to new avenues for control of TB.

Keywords: BCG, epigenetics, innate immunity, trained immunity, tuberculosis.

Introduction

Mycobacteria belong to one of the largest and most ancient phyla of bacteria, the Actinobacteria [1], which occupies various habitats on Earth including soil and water. Most mycobacteria are free-living, harmless saprophytes, but a few species have evolved as pathogens that infect a variety of species. Although pathogenic mycobacteria are not strictly species specific, *Mycobacterium tuberculosis* [which causes tuberculosis (TB)], *Mycobacterium leprae* (which causes leprosy), and *Mycobacterium ulcerans* (which causes painless skin lesions known as Buruli ulcers) are considered primarily human pathogens. *M. tuberculosis* was one of the first bacteria found to be the causative agent of a disease and is still among the most deadly human pathogens, causing approximately 1.4 million deaths per year. TB cannot be eliminated with the current tools of prevention, diagnosis, and treatment [2] and thus novel strategies to fight the disease are urgently needed. During the last decade, many advances in the understanding of the complex biology of TB have been made. However, these efforts have not yet resulted in an improved regimen for TB control, such as a more effective vaccine or a shorter treatment duration, with the standard regimen

being at least 6 months with a combination of between two and four antibiotics.

In the proposed checkpoint model for TB disease progression [3], the innate immune system constitutes the first ‘checkpoint’ that *M. tuberculosis* has to pass to establish infection in a host (Fig. 1). Only by passing the second ‘checkpoint’, which is represented by adaptive immunity acting in concert with innate immunity, is the bacterium able to cause active disease and spread to other individuals. Though adaptive immunity undoubtedly plays a central role in control of the infection in individuals in whom *M. tuberculosis* has established itself, the model suggests that there is a window of opportunity during which innate immune mechanisms can eliminate *M. tuberculosis*. Indeed, there is substantial evidence that many individuals who live under constant exposure, e.g. household contacts of TB cases, can clear the infection without the involvement of adaptive immunity. This is referred to as ‘early clearance’ and can be explained by the presence of a highly effective innate immunity in these individuals [4].

It has been suggested that the effectiveness of these two immune barriers depends on genetic [5]

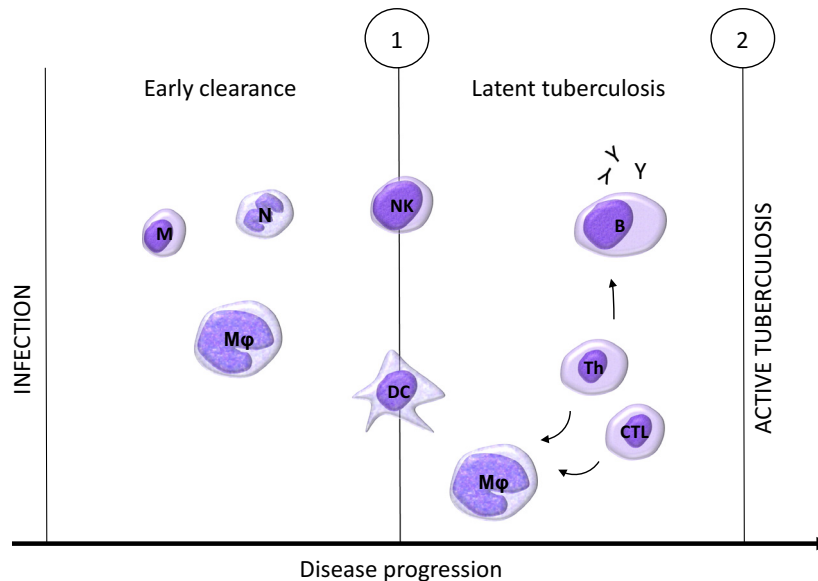


Fig. 1 The checkpoint model for tuberculosis (TB) disease progression. Innate immunity poses the first 'checkpoint' (1) that *Mycobacterium tuberculosis* has to pass to cause disease. At the second 'checkpoint', *M. tuberculosis* also has to overcome the contribution of adaptive immunity (2). Here, control or elimination of the pathogen through innate immunity is referred to as early clearance. Latent TB represents the dynamic equilibrium between adaptive immunity and *M. tuberculosis*. NK, natural killer cell; DC, dendritic cell; Th, T helper cell; CTL, cytolytic T cell; B, B cell; M, monocyte; N, neutrophil; M ϕ , macrophage.

and environmental factors, such as standard of living (nutritional state and housing), and comorbidities [6, 7]. Another, not yet fully understood, environmental factor in this context is the impact of previous exposure of innate immunity to microbial agents or even vaccines, which could educate the innate immune defence to pose a more effective response against related and sometimes even unrelated infections. This evolutionarily conserved phenomenon is known as trained immunity and has been demonstrated in plants, invertebrates, and animals [8].

Here, we will review the immunological mechanisms that are important for control of TB and discuss how trained immunity can contribute to control and elimination of mycobacterial infections.

The life cycle of *M. tuberculosis*

The primary target cells for *M. tuberculosis* infection are the alveolar macrophages. These cells encounter and engulf the pathogen when infected aerosols are inhaled and enter the alveolar space (step 1, Fig. 2). *M. tuberculosis* employs several

mechanisms to counteract the microbicidal activities of these cells, which include phagosomal acidification, activation of proteolytic enzymes in acidified phagolysosomes, and production of antimicrobial peptides as well as reactive oxygen and nitrogen metabolites [9, 10]. Thus, if the macrophage defence fails, *M. tuberculosis* establishes itself in an intracellular niche, which is initially the phagosome with impaired antimicrobial capacity [11, 12] and later the cytosol [13] into which it escapes by means of a membrane-damaging toxin, early secreted antigenic target (ESAT)-6 [14]. The cytosolic mycobacteria replicate efficiently (step 2, Fig. 2) before the primary host cell is killed (step 3, Fig. 2), a process in which ESAT-6 plays an important role [15]. The inflammation triggered by the dying cells induces recruitment to the site of infection of further monocytes/macrophages (step 4, Fig. 2), which then become infected (step 5, Fig. 2). Paradoxically, these cells can serve as vehicles for further dissemination of the infection in the tissue [16]. The process of early granuloma formation is also dependent on ESAT-6 [16, 17] and is probably linked to the inflammation generated through necrosis triggered by the toxin [15]. As the inflammatory process proceeds, gran-

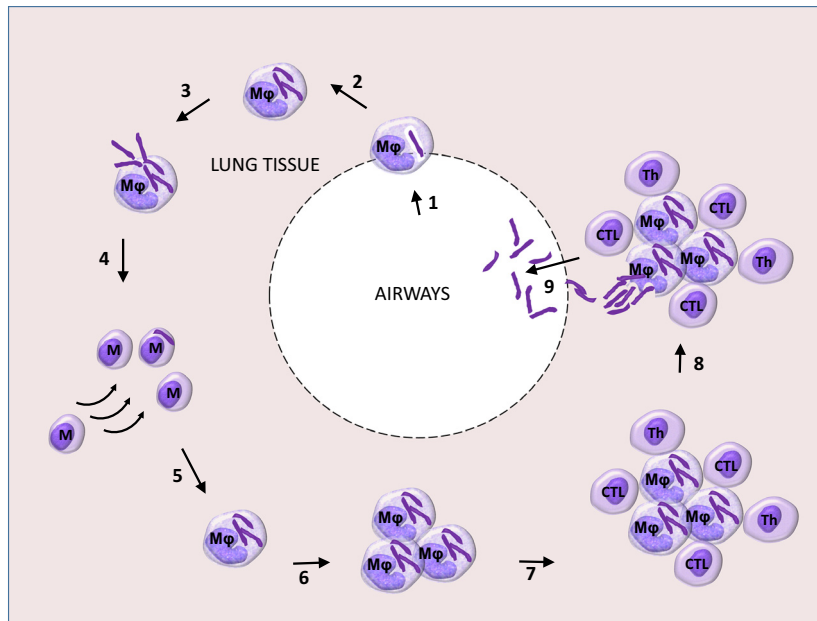


Fig. 2 The life cycle of *Mycobacterium tuberculosis*. *M. tuberculosis* is taken up by alveolar macrophages (Mφ) (step 1) and replicates efficiently (step 2) until the host cells are killed (step 3). Recruitment of further monocytes (M)/macrophages to the site of infection (step 4) disseminates the infection (step 5) and as the inflammatory process proceeds, granuloma are formed (step 6). CD4⁺ T helper type 1 (Th1) cells and CD8⁺ cytolytic T cells (CTLs) are recruited to form a rim surrounding the infected macrophages (step 7). Eventually, the granuloma becomes necrotic (step 8) and the structure ruptures, draining viable bacilli into the alveolar space (step 9).

ulomas (clusters of macrophages) are formed (step 6, Fig. 2). As the granuloma further matures, lymphocytes, including both CD4⁺ T helper type 1 (Th1) cells and CD8⁺ cytolytic T cells (CTLs) [18, 19], are recruited to form a rim surrounding the infected macrophages (step 7, Fig. 2). Eventually, the inner core of the granuloma becomes necrotic (step 8, Fig. 2) and the structure ruptures. Drainage of viable bacilli into the alveolar space (step 9, Fig. 2) completes the life cycle of *M. tuberculosis* and aerosol-producing cough will spread the pathogen to other individuals.

The immunological barriers opposing *M. tuberculosis* infection

Although very few *M. tuberculosis* bacteria are required to cause infection, only 5–10% of infected individuals develop active TB. Most individuals (65–75%) who are infected maintain a state referred to as latent TB (Fig. 1). Latent TB is clinically defined as the presence of an immunological memory of exposure to *M. tuberculosis* (as can be proven by immunological tests) in individuals who do not display any clinical signs of disease and are not contagious. It is interesting

that a relatively large proportion (20–25%) of individuals who are exposed to TB never develop any signs of an immunological memory against *M. tuberculosis*, suggesting that the high efficacy of the innate immune response in these individuals precludes the need for adaptive immunity. This phenomenon is termed early clearance [4] (Fig. 1). Below we will discuss the mechanisms of latent TB and early clearance.

Control of *M. tuberculosis* infection during latent tuberculosis

Inside TB granulomas, innate immunity acts in alliance with adaptive immunity to control *M. tuberculosis* infection. The immunological mechanisms that contribute to this dynamic equilibrium include activation of macrophages via pro-inflammatory cytokines released by Th1 cells and the activity of CTLs (reviewed in [19]). Thus, infected macrophages can be either stimulated to more effectively kill intracellular *M. tuberculosis* or killed along with the pathogen through the action of CTLs. Although *M. tuberculosis* is mainly regarded as an intracellular pathogen, parts of its life cycle are extracellular. In recent years, the importance of

antibody-mediated protection against TB has been highlighted, and there is evidence for protective B-cell-mediated immunity (reviewed in [20]). These adaptive immune mechanisms are established through the presentation of mycobacterial antigens by dendritic cells (DCs) in the lymph nodes. Antigen presentation in the case of TB can occur via both MHC class I (triggering CTL responses) and MHC class II (triggering a Th-cell response) [21].

The role of natural killer (NK) cells in the protection against TB is less well understood (reviewed in [22]), and though it is clear that NK cells can kill infected monocytes [23], the anti-mycobacterial effect mediated by these cells remains unknown [24].

Of note, recent studies have shown that granulomas are highly dynamic structures that can grow and shrink and that individual granulomas in the same tissue can have different fates [25]. This suggests that local rather than systemic immunological mechanisms determine the outcome (clearance of bacteria or excessive necrosis) in each granuloma. Thus, it is possible that tissues direct the fate of the infection locally. This is in line with evidence showing that local factors in the tissue direct macrophage differentiation and modulate macrophage function [26].

Although there is a brief period during which the adaptive immune mechanisms active in the granuloma clearly limit the progression of TB, the life cycle of *M. tuberculosis* inevitably involves and depends on the granuloma structure, as the bacterium disseminates to other hosts via rupture and drainage of the necrotic core of mature granulomas. Thus, the dogmatic view of the granuloma as a host-protective structure has been a matter of debate during recent years (reviewed in [27]). As mentioned above, recent studies show that the most prominent virulence factor of *M. tuberculosis*, ESAT-6, is required for early granuloma formation [16, 17], and the dissemination of infection in recently infected tissue is facilitated by this process. Thus, *M. tuberculosis* actively induces granuloma formation. It has been suggested that the induction of necrosis in mature granulomas depends on Th1 responses [28], and it is plausible that *M. tuberculosis* has diverted the host immune response to its own advantage for this critical step in its life cycle. Support for this notion comes from the fact that T-cell epitopes of *M. tuberculosis* are hyperconserved, i.e. have been evolutionarily

subjected to a lower mutation frequency than the metabolic enzymes of the bacterium [29]. This finding has received much attention in the field, leading to the question: how can an effective vaccine be developed that would be active against an intracellular pathogen that could have evolved to be presented to T-cell-mediated immunity?

The concept of early clearance of *M. tuberculosis* infection

Increased understanding of the mechanisms underlying the inherent resistance towards TB as observed in 'early clearers' may provide novel opportunities for improved strategies for TB prevention. Early clearance of TB has been reported in a number of studies, one of the most cited being the report of an outbreak on a US naval ship where 13 exposed individuals did not develop any signs of exposure, i.e. had a negative tuberculin skin test reaction after 6 months [30]. This suggests that the protected individuals were able to mount an effective innate immune response without the involvement of adaptive immunity.

Many studies have addressed genetic susceptibility to TB, and aberrant polymorphisms/mutations in the genes encoding natural resistance-associated macrophage protein (NRAMP) 1, interferon (IFN)- γ receptors, and toll-like receptor (TLR) 2 are examples of genetic factors that confer TB susceptibility (reviewed in [31]). On the other hand, genetic variations that may confer enhanced resistance to TB include combined polymorphisms leading to increased production of IL-1 β [32], heterozygosity for leukotriene A4 hydrolase polymorphisms [33], and a polymorphism of the vitamin D receptor [34].

An alternative explanation for the phenomenon of early clearance is epigenetic reprogramming of innate immune cell function, with an increased capacity to eliminate pathogens. This alternative phenotype of monocytes, macrophages or NK cells can be induced by a previous vaccination, colonization or even infection, and displays stronger activation in response to a second exposure to microbial molecules. This is accompanied by histone modifications that facilitate gene transcription, with the priming effect on innate immunity lasting for up to 1 year [35]. The finding provides new understanding of innate immunity, which, contrary to previous views, may acquire adaptive characteristics and an increased response to secondary infections or stimuli. This phenomenon has been termed trained immunity (Fig. 3). Below we

will consider how trained immunity is induced and discuss how it could be exploited for the development of a novel strategy to control TB.

How does innate immunity change during the lifetime of an individual?

There is a substantial difference in the levels of cytokines and chemokines produced by lipopolysaccharide-stimulated monocytes obtained from adult blood as compared to monocytes from umbilical cord blood [36], indicating that monocyte responsiveness to microbial stimuli is enhanced during an individual's lifetime. In support of this, analysis of the DNA methylome of foetal and maternal myeloid cells revealed that a number of immunity-related genes were hypermethylated (inactive) in foetal cells as compared to the corresponding DNA methylome of maternal monocytes, suggesting that the monocytes of newborn babies are in a 'reset mode' and thus likely to mount a more moderate immune response upon the first encounter with microorganisms [37]. The impact of external factors on lifetime monocyte/macrophage function was recently shown in a large twin study, in which the relative contribution of environmental and hereditary factors to the heterogeneity of the immune system (including adaptive and innate immune components) was investigated. The results of the study showed that early in life the

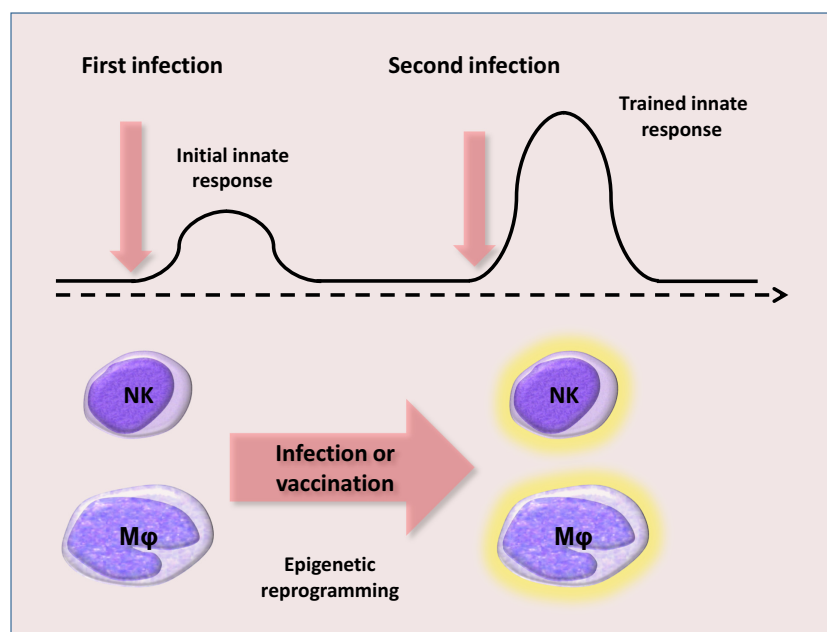
immune responses of homozygotic twins are more similar to each other than at older ages, when the variability increases [38]. The authors concluded that this immune divergence is due to epigenetic effects of environmental factors such as infections, vaccinations, or other types of exposure (e.g. to the microbiome), with 80% of the immune phenotype of an adult individual due to environmental influences and a hereditary contribution of only 20%.

Microbial colonization of the human mucosal tissues and skin occurs at birth, with the first exposure to the vaginal flora during delivery. In healthy individuals, the diversity of the microbiome increases during the first years of life, and recent studies have shown that children with low-diversity flora are at higher risk of dysregulated immune function [39, 40]. In fact, the microbiome plays a pivotal role in the normal development of the immune system, as studies of germ-free mice have shown that several immunological structures such as secondary lymph nodes are under-developed in the absence of gut microbiota [41].

What is the molecular substrate of innate immune memory?

In multicellular organisms, all diploid cells share the same DNA and the phenotype of the individual cell types is directed by cell type-specific epigenetic modifications. These alterations include DNA

Fig. 3 The concept of trained immunity. Innate immune cells are epigenetically reprogrammed during exposure to microorganisms or vaccines, allowing an enhanced response upon a second exposure. NK, natural killer cell; M ϕ , macrophage.



methylation and histone modifications, such as acetylation, methylation, and phosphorylation, and are determinants of which genes are silent and which are actively transcribed, thus influencing the traits of the individual cell types. Although the underlying chemistry of DNA methylation is totally different from that of histone modifications, it seems that these processes are interdependent [42]. During differentiation of blood cells, highly specific sequential changes in the pattern of epigenetic modifications direct the genesis of different cell types [43]. There are several lines of evidence demonstrating that epigenetic traits induced by environmental cues such as lifestyle and episodes of hunger in the parental generation can be transferred to the offspring [44, 45]. Dysregulation of epigenetic modifications has been extensively studied in the context of cancer, but little is known how exposure to microorganisms affects the epigenome of humans and animals. Acquired resistance to infections has been established in plants and the fruit fly, both of which lack classical adaptive immunity [46, 47]. Indeed, recent studies in plants and insects suggest that exposure of the parental generation to microbial infection or microbial stimulation can result in enhanced protection of the offspring to infections [46, 48–51], and modulation of DNA methylation patterns has been considered a possible molecular mechanism for this trans-generational immune protection.

In humans, it has been shown that trained immunity is mediated by epigenetic reprogramming through modulation of histone modifications such as methylation and acetylation that are able to control chromatin accessibility and gene transcription. In the case of mycobacteria, the trained immunity response is induced via nod-like receptors (NLRs), which are pattern recognition receptors (PRRs) that are expressed in the cytoplasm of immune cells. NLRs respond to intracellular pathogen-associated molecular patterns, thereby stimulating intracellular pathways that enhance antimicrobial functions such as autophagy (triggered by Nod1 and Nod2 [52]) and production of the pro-inflammatory cytokine interleukin-1 β (triggered by NLRP3 [53]). A second consequence of Nod2 activity is the functional reprogramming of monocytes through epigenetic changes at the level of chromatin status [54, 55]. Another PRR, Dectin-1, responds to β -glucan, a component of fungal cell walls, by inducing training of monocytes. It was found that the training effect is accompanied by increased chromatin accessibility at the level of

both promoters (histone H3 lysine⁴ trimethylation and histone H3 lysine²⁷ acetylation) and enhancers (histone H3 lysine⁴ monomethylation), leading to an increased gene transcription pro-inflammatory cytokines such as tumour necrosis factor- α and IL-6 after cell restimulation [56, 57]. Subsequently, the inhibition of histone methyltransferases or acetyl transferases blocked these effects, demonstrating a role of this epigenetic-based mechanism for induction of innate immune memory [56, 57].

Pathway analysis of the transcriptome and epigenome profiles of trained monocytes has also demonstrated a central role for the rewiring of cellular metabolism, with the mTOR pathway and glycolysis being crucial for the induction of trained immunity [58]. As in activated lymphocytes, a shift in glucose cellular metabolism from oxidative phosphorylation towards aerobic glycolysis (known as the Warburg effect) was necessary for the function of trained cells [58].

In conclusion, trained immunity is mediated by epigenetic reprogramming resulting in changes in the transcriptional programme of innate immune cells, with both immunological and metabolic pathways targeted in this process.

The enigmatic mode of action of the most commonly used vaccine, the TB vaccine

In the 1920s, two French scientists, Drs Albert Calmette and Camille Guérin, developed a TB vaccine known as *Bacillus Calmette–Guérin* (BCG), which is currently given as an intradermal injection to newborn babies according to the WHO recommendations. BCG is an attenuated strain of *M. bovis* that has lost its region of difference encoding the genes for ESAT-6 and the apparatus by which this toxin is secreted [59].

Bacillus Calmette–Guérin has been shown to protect against disseminated forms of TB such as TB meningitis and miliary TB in children [60]. However, its efficacy in protecting against pulmonary TB is low (below 30%) and highly variable depending on the study cohorts [60]. Because vaccination programmes are by far the most effective way of preventing infectious disease, a number of studies and clinical trials have been undertaken during the last decade to identify a vaccine that has better efficacy than BCG. Two of the most extensive recent clinical trials assessed the safety and effi-

cacy of MVA85A in healthy newborn babies [61] and HIV-positive adults [62]. Unfortunately, although the vaccine proved safe in both cohorts, the MVA85A/BCG regimen was not superior to BCG alone in terms of efficacy. MVA85A is a vaccinia virus-based vaccine in which the *M. tuberculosis* antigen 85A is added to the standard BCG vaccine. The rationale behind this and many other TB vaccine candidates is to add an *M. tuberculosis* antigen to BCG to elicit a stronger adaptive immune response to the vaccination. Of note, the induction of adaptive immunity in response to a vaccine or an infection does not necessarily mean that the induced immunological activities are protective. BCG vaccination triggers adaptive immunity to respond to stimulation with mycobacterial antigens by inducing T cells to produce IFN- γ . It is clear that IFN- γ plays a crucial role in host protection against *M. tuberculosis*, as patients suffering from deficiencies in the IFN- γ signalling network are highly susceptible to mycobacterial infection [63]. It has been shown that IFN- γ , at least in the mouse, stimulates the anti-mycobacterial capacity of macrophages [64]. However, T cells are not the only source of IFN- γ , which can also be produced by innate immune cells such as NK cells [65] and in smaller amounts even by macrophages [66].

A large study of BCG-vaccinated children to assess whether mycobacteria-specific T-cell responses were higher in those who did not develop active TB, failed to prove a correlation between the studied parameters [67]. One explanation for this finding could be that BCG triggers a nonprotective, adaptive immune response and, at the same time, a protective response due to trained immunity, as has been demonstrated in monocytes and NK cells [54, 55]. Below we will describe the effects of BCG from the perspective of innate immunity.

Is BCG-induced protection against mycobacterial infections due to trained immunity?

Bacillus Calmette–Guérin has long been known to have preventive effects against diseases other than TB. The first observation of such heterologous immunity came from a Swedish study published in 1931, which demonstrated BCG-induced protection against childhood mortality due to infections other than TB [68]. Since then, similar observations have been made in many studies, including more recent studies conducted in West Africa [69], Papua New Guinea [70], Bangladesh [71], and India [72]. We recently demonstrated heterologous innate immunity induced by BCG in a mouse model of candida infection [54]. SCID mice, which lack adaptive immunity, are very sensitive to *Candida albicans* infection, however, the mice were protected if given an intradermal injection of BCG vaccine prior to challenge with a lethal inoculum of *C. albicans*. This finding suggests that the mice were protected against the fungal infection through trained immunity induced by the bacterial components of BCG. In this study as well as in our more recent work, we have shown that BCG induces an increased responsiveness of human monocytes and NK cells to secondary stimulation and that this effect is due to increased histone H3 lysine⁴ trimethylation via the NOD2 pathway [54, 55]. These findings support the notion that innate immunity can have adaptive features in that a stronger response is elicited upon a second stimulus. These studies also provide evidence that epigenetic changes such as modifications of histones are the mechanism by which innate immunity can remember previous exposure to pathogens.

As proposed above, an effective innate immune response could explain the phenomenon of early

Table 1 Summary of the discussed clinical and experimental observations of trained immunity

Observation of trained immunity	Observational characteristics	Ref. No.
US naval ship outbreak (TB)	No TST reaction in a number of Mtb-exposed individuals	[30]
Adult vs perinatal monocytes	Increasing monocyte responsiveness during lifetime	[36]
Twin study on immunity	The innate immune response of twins diverges over time	[38]
BCG-induced epigenetic memory	Enhanced function of monocytes and NK cells after BCG vaccination	[54, 55]
Reduced childhood mortality	BCG vaccination reduces childhood mortality (not only TB-related)	[68–71]
Protection against <i>Candida</i>	BCG-vaccinated SCID mice are protected against <i>Candida</i> infection	[54]
Monocytes trained by β -glucan	β -glucan epigenetically reprograms monocytes	[57, 58]

TB, tuberculosis; TST, tuberculin skin test; NK, natural killer cell; BCG, Bacillus Calmette–Guérin.

clearance of TB infection, and experimental and translational studies should be performed to investigate the possibility that trained immunity may explain the BCG-induced protection against TB. This would provide important information about how a more effective TB vaccine could be designed.

Concluding remarks

Based on the studies reviewed above (summarized in Table 1), it has become increasingly evident that both adaptive and innate immunity mechanisms are important for host defence against TB. The emerging view is that innate immunity can be trained, i.e. to enhance its response through exposure to microorganisms and thus display adaptive features. This phenomenon could be an important factor for early clearance of TB infection and also provide an explanation for the protective effects of BCG. The challenge in the coming years will be to decipher the details of the molecular and immunological mechanisms of trained immunity induced by BCG, to develop novel and improved vaccination strategies that would more effectively induce both classical adaptive immune memory and trained immunity against TB.

Conflict of interest statement

No conflicts of interest were declared.

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