Clinical & Translational Immunology

Australian and New Zealand Society for Immunology Inc.

Clinical & Translational Immunology 2020; e1228. doi: 10.1002/cti2.1228
www.wileyonlinelibrary.com/journal/cti

REVIEW

Trained immunity as a novel approach against COVID-19 with a focus on Bacillus Calmette–Guérin vaccine: mechanisms, challenges and perspectives

Yahya Sohrabi[1,2], Jéssica Cristina Dos Santos[3], Marc Dorenkamp[1], Hannes Findeisen[1], Rinesh Godfrey[1], Mihai G Netea[3,4] & Leo AB Joosten[3,5]

[1]Department of Cardiology I – Coronary and Peripheral Vascular Disease, Heart Failure, University Hospital Münster, Münster, Germany
[2]Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
[3]Department of Internal Medicine and Radboud Centre of Infectious Diseases (RCI), Radboud University Medical Centre, Nijmegen, The Netherlands
[4]Department for Genomics & Immunoregulation, Life and Medical Sciences Institute (LIMES), University of Bonn, Bonn, Germany
[5]Núcleo de Pesquisa da Faculdade da Polícia Militar (FPM) do Estado de Goiás, Goiânia, Brazil

Correspondence
Y Sohrabi, Department of Cardiology I – Coronary and Peripheral Vascular Disease, Heart Failure, University Hospital Münster, Münster, Germany; Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic.
E-mail: yahya.sohrabi@ukmuenster.de

LAB Joosten, Department of Internal Medicine and Radboud Centre of Infectious Diseases (RCI), Radboud University Medical Centre, Nijmegen, The Netherlands; Núcleo de Pesquisa da Faculdade da Polícia Militar (FPM) do Estado de Goiás, Goiânia, Goiás, Brazil.
E-mail: Leo.Joosten@radboudumc.nl

Received 20 May 2020;
Revised 3 September, 15 October and 29 November 2020;
Accepted 29 November 2020

doi: 10.1002/cti2.1228

Clinical & Translational Immunology **2020; 9: e1228**

Abstract

COVID-19 is a severe health problem in many countries and has altered day-to-day life in the whole world. This infection is caused by the SARS-CoV-2 virus, and depending on age, sex and health status of the patient, it can present with variety of clinical symptoms such as mild infection, a very severe form or even asymptomatic course of the disease. Similarly to other viruses, innate immune response plays a vital role in protection against COVID-19. However, dysregulation of innate immunity could have a significant influence on the severity of the disease. Despite various efforts, there is no effective vaccine against the disease so far. Recent data have demonstrated that the Bacillus Calmette–Guérin (BCG) vaccine could reduce disease severity and the burden of several infectious diseases in addition to targeting its primary focus tuberculosis. There is growing evidence for the concept of beneficial non-specific boosting of immune responses by BCG or other microbial compounds termed trained immunity, which may protect against COVID-19. In this manuscript, we review data on how the development of innate immune memory due to microbial compounds specifically BCG can result in protection against SARS-CoV-2 infection. We also discuss possible mechanisms, challenges and perspectives of using innate immunity as an approach to reduce COVID-19 severity.

Keywords: BCG, COVID-19, trained immunity, vaccine

PATHOLOGY AND IMMUNOLOGY OF COVID-19

The coronavirus disease 2019 (COVID-19) is caused by an RNA virus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease became a major health problem very quickly, since December 2019 when it was reported for the first time in Wuhan, China.[1] The disease COVID-19 was declared a pandemic disease by the

World Health Organization in March 2020. According to Johns Hopkins University, as of November 29th 2020, the number of infected cases exceeded 62.5 million, with almost 1.5 million deaths worldwide (https://coronavirus.jhu.edu/map.html).

The outbreak was initiated as a zoonotic disease and later spread through human-to-human transmission.[2] Clinical symptoms of the disease are relatively mild, beginning with fever, dry cough and dyspnoea and possibly followed by headache and generalised fatigue.[3] The incubation period of COVID-19 is relatively long during which patients are highly contagious. The coronavirus infection can severely develop and become potentially lethal as a result of pneumonia and multiple organ failure.[3] High mortality is seen in the elderly and those with underlying medical conditions such as hypertension, diabetes, cardiovascular disease, COPD (chronic obstructive pulmonary disease), cancer, immunocompromised states and chronic kidney disease or in conditions such as obesity, pregnancy and smoking.[4,5]

Angiotensin-converting enzyme 2 (ACE2) mediates entry of SARS-CoV-2 into the host cells. ACE2 is expressed in the lungs, heart, arteries, kidney and intestinal tissue.[6] The expression of ACE2 is higher in respiratory epithelial cells; however, it is also expressed in alveolar macrophages, dendritic cells, innate lymphoid cells and natural killer (NK) cells.[7] ACE2 is also heavily expressed in endothelial cells of infected patients. Thus, injury of these cells could facilitate virus invasion.[8]

A significant increase of C-reactive protein (CRP) levels was correlated with poor prognosis in COVID-19 patients.[9] Increased CRP level upregulates CD32 expression on monocytes, neutrophils, human aortic endothelial cells and kidney tubular epithelial cells.[10] In addition, alveolar macrophages generally express high levels of CD32.[11] Interestingly, individuals with the previously mentioned underlying medical conditions such as diabetes and cardiovascular diseases express high levels of CD32 on their innate immune cells[12,13] due to elevated C-reactive protein (CRP) level. CD32 on monocytes or macrophages can transduce inflammatory signalling in the presence of IgG and result in the production of IFN-γ, TNF-α, IL-1β and IL-6.[11] It was recently shown that SARS-CoV could use the CD32 receptor to enter the cells via the so-called Trojan horse mechanism.[14]

SARS-CoV-2 infection increases the production of pro-inflammatory cytokines including IL-6, MCP1, G-CSF, MIP1A, TNF-α and GM-CSF,[3,15] which was closely correlated with the disease severity. Intensive care patients are characterised by a strong systemic inflammatory response, whereas the level of inflammatory mediators was lower in patients with mild symptoms.[15] Epithelial cells are one of the major cells involved in SARS-CoV-2 infection. Infected lung epithelial cells produce IL-8, which is a well-known chemokine that recruits neutrophils and T cells.[16] In response to RNA and dsRNA viruses, toll-like receptor 3 (TLR3) promotes antiviral activity through the production of pro-inflammatory cytokines and the upregulation of the adhesion molecule ICAM-1 (intercellular adhesion molecule 1) in primary human bronchial epithelial cells.[17] SARS-CoV and possibly SARS-CoV-2 activate TLR3 and TLR4, subsequently inducing an inflammatory reaction, inflammasome activation and upregulation of the IL-1β pathway.[18] The number of inflammatory monocytes was significantly increased in COVID-19 patients, which might be the primary producer of IL-6 and responsible for inducing the cytokine release syndrome.[7,19] This finding postulated the usage of neutralising or inhibiting antibodies against IL1 and IL-6 for treating the severe form of infection.[20]

Over the last few months, enormous efforts have been put into place in many countries across the world in order to find an effective vaccine against COVID-19. It is expected that it may take at least 12–18 months to produce a first vaccine against the disease.

Severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) had low incidence rates, which made their control possible and achievable. Nevertheless, low incidence and limited geographical spread lowered the commercial interest to invest in developing vaccines against these diseases.[21] The number of unidentified and undocumented cases with SARS-CoV-2 infection is very high due to mild, limited, or a complete lack of symptoms, thereby accelerating the transmission rate and thus the number of deaths. Current knowledge about COVID-19 is very limited and whether long-term protection can be achieved post-coronavirus exposure is not yet clear. Recent studies have suggested that COVID-19 incidence and mortality rate is lower in countries where the BCG vaccine is a part of the routine childhood immunisation

schedule.[22–24] The non-specific cross-protection against unrelated diseases is not limited to BCG and have also been described for other vaccines such as influenza.

CROSS-PROTECTION OF MICROBIAL COMPONENTS AND HUMAN VACCINES

An increasing number of studies have described the non-specific protective effects against diseases after immunisation with an unrelated vaccine or microbial antigen. This could be an approach used to fight COVID-19. This de facto immunological memory occurs in innate immune cells or non-immune cells and has been termed 'trained immunity'. Recent studies show, that innate immune responses have adaptive characteristics that can lead to protection against subsequent unrelated infections. As a proof of concept, studies performed in plants and invertebrates, which are organisms lacking adaptive immune response, have shown that both can develop immunological memory upon infection.[25,26] The innate immune response is mainly composed of monocytes, macrophages, neutrophils and NK cells, which act rapidly and non-specifically after an infectious agent or toxin is encountered. Interestingly, recent studies have implicated that innate immune memory is not exclusively confined to immune cells, but non-immune cells including human coronary smooth muscle,[27] stromal and epithelial[28,29] cells can also develop immunological memory.

Several studies using mouse and human cells have demonstrated that the exposure of innate immune cells to *Candida albicans* or the fungal cell wall component β-glucan, as well as to certain mammalian models of vaccination or metabolites can lead to the development of what we know as 'innate immune memory' or 'trained immunity' (Table 1). In contrast to adaptive immune responses, the innate immune memory is not strictly specific, as infection with one infectious agent often also protects from another unrelated microorganism. This non-specific memory leads to enhanced capacity of immune cell response to reinfection.[30–32]

The induction of trained immunity by β-glucan is known to confer non-specific protection from subsequent bacterial and parasitic infections caused by *Staphylococcus aureus* and *Leishmania braziliensis*, respectively.[33,34] Similarly to β-glucan, muramyl dipeptide (MDP), a component of bacterial cell wall peptidoglycan, can induce protection against toxoplasmosis,[35] while CpG nucleotides provide protection against *Escherichia coli* infections.[36] Intranasally administered TLR3 ligands were shown to induce protection against genital Herpes simplex virus (HSV-2) infection.[37]

In addition to microbial components, trained immunity can be induced through the exposure to vaccines. Vaccines are usually made to protect against infectious diseases and are known to have specific effects. However, during the past few decades, the non-specific effects exerted through vaccines have been described in studies from low-income countries with high infectious disease incidence, such as West Africa as well as in a high-income populations for example Copenhagen, Denmark. Randomised trials have shown that measles, Bacillus Calmette-Guérin (BCG) (tuberculosis) and Vaccinia (smallpox) vaccine were associated with a reduction in overall child mortality and morbidity. This effect was mainly due to a decrease in lower respiratory tract infections and sepsis, which cannot be explained by prevention of the target disease itself.[38–40] Additional studies in Africa have shown similar non-specific beneficial effects of BCG against respiratory infections and pneumonia in children.[41,42] Importantly, BCG has shown a protective effect against pneumonia in older adults.[43] Some studies suggest that BCG and Vaccinia may exert beneficial non-specific effects against melanoma and non-Hodgkin lymphoma.[44,45]

The BCG vaccine is one of the most used vaccines worldwide. Furthermore, a growing body of evidence associates its non-specific protective effects with the induction of trained immunity.[46] The clinical relevance of trained immunity has been presented in a randomised placebo-controlled study demonstrating that BCG vaccination reduces yellow fever viraemia.[47] Moreover, BCG has been shown to offer protection against malaria,[48] *Leishmania* species,[49,50] *Candida albicans*[46] and Influenza virus.[51] Beneficial effects of recombinant BCG vaccine against viral diseases such as hepatitis C virus (HCV), human immunodeficiency virus (HIV) and measles have also been reported.[52–54] These antiviral effects were attributed to an induction of humoral and cell-mediated immune response to HIV infection and to the development of specific cytotoxic T lymphocytes in HCV infection.[53,54] It was also shown that the BCG

Table 1. Non-specific beneficial effects of microbial components and human vaccines

Compound	Cross-protection	Study type and model	Reference
Microbial compounds			
β-glucan	*Staphylococcus aureus*	Mice	34,118
Muramyl dipeptide (MDP)	*Toxoplasma gondii*	Mice	35
CpG	*Escherichia coli*	Mice	36
Poly(I:C)	Genital HSV2	Mice	37
Poly(IC: LC)	SARS	Mice	119
MDP-Lys (L18)	Sendai virus	Mice	120
MTP-PE	Influenza viruses A and B		121
β-glucan	*L. (Viannia) braziliensis*	Human cells (*in vitro* and *ex vivo*) and mice	33
β-glucan	sepsis	Human cells (*in vitro* and *ex vivo*)	64,122
Vaccine			
BCG	*L. major*	Mice	50
BCG	*C. albicans*	Mice	46
BCG	Influenza A	Mice	55
BCG	HSV1	Mice	123
Measles	Respiratory infections	Randomised controlled trials	39
Vaccinia (smallpox)	Reduced childhood mortality	Case–cohort study	38
Vaccinia (smallpox)	Melanoma	Cohort study	44
Polio	Non-Hodgkin lymphoma	Population-based, case–control study	45
Smallpox	Non-Hodgkin lymphoma	Population-based, case–control study	45
M.M.R.	Lower respiratory infections	Cohort study	124,125
MMR	RSV infection	Cohort study	126
OPV	Lower respiratory infections	Cohort study	127
BCG	Sepsis, reduced childhood mortality	CASE–cohort study	38
BCG	Pneumonia and sepsis	Randomised controlled trials	42
BCG	respiratory tract infections	randomised controlled trials	41
BCG	Pneumonia in elder	Clinical trial	43
BCG	Melanoma	Cohort study and case–control study	44
BCG	Yellow fever viraemia	Randomised placebo-controlled study	47
BCG	*Plasmodium falciparum*	Randomised controlled study	48
BCG	*L. amazonensis*	Human case report	49
BCG	Influenza virus	Randomised, Placebo-Controlled trial	51
BCG	Bladder cancer	Human *in vitro* and in vivo	56
MTBVAC	Pneumonia	Human *in vitro*, mice *in vivo*	128
Live virus			
Epstein–Barr virus	Bacterial infection	Mice	57
Cytomegalovirus	Bacterial infection	Mice	57
Adenovirus infection	Bacterial infection	Mice	129
Hepatitis B	Bacterial infection	Human *in vitro*	58

vaccine boosts efferocytosis within the alveolar space in mice and protects the host against influenza pneumonia by rapidly removing apoptotic cells, maintaining lung homeostasis and reducing inflammation.[55] Remarkably, BCG-induced potent non-specific effects during bladder cancer therapy have also been described and is now used in routine therapy of the early stages of disease.[56]

Aside from vaccines, exposure to viruses has also been shown to induce non-specific effects. Latent infections with Epstein–Barr virus or cytomegalovirus were shown to confer protection in mice against bacterial infection.[57] Moreover, Hepatitis B exposure in utero exhibited trained immunity features, which enhances the ability of cord blood immune cells of newborns to respond to bacterial infection *in vitro*.[58]

UNDERLYING MECHANISMS OF CROSS-PROTECTION IN TRAINED IMMUNITY

The induction of trained immunity is mediated by functional, metabolic and epigenetic reprogramming of innate immune cells, which,

contributes to an enhanced immunological response to subsequent pathogenic encounters.[26]

Studies using Rag1 (recombination-activating gene 1)-deficient mice and mice with severe combined immunodeficiency (SCID) revealed that the beneficial effects of trained immunity are exclusively carried out by innate immune cells. After priming with either β-glucan or BCG, these mice were protected (improved survival) from reinfection by *C. albicans* or *S. aureus*. These data revealed that both T- and B-lymphocytes are not involved in the trained immune phenotype displayed by these animals. Furthermore, *C. albicans*-primed CCR2 (chemokine receptors 2)-deficient mice were found to be susceptible (reduced survival) to reinfection, which demonstrated the importance of monocytes and macrophages in promoting trained immunity.[30,46]

The functional modulation of monocytes induced by β-glucan occurs through dectin-1 and the noncanonical serine-threonine kinase Raf-1 pathway, whereas BCG effects occur in a NOD2 (nucleotide-binding oligomerisation domain-containing protein 2)-dependent manner. The interaction of β-glucan or BCG with dectin-1 and NOD2 respectively leads to increased production of monocyte-derived cytokines such as TNFα and IL-6 in response to *in vitro C. albicans*, *S. aureus*, *Mycobacterium tuberculosis* and lipopolysaccharide (LPS) re-stimulation.[30,46] Moreover, a placebo-controlled human challenged study showed that BCG vaccination lowers yellow fever vaccine-induced viraemia through the induction of IL-1β production. The non-specific IL-1β production conferred by BCG vaccination was associated with protection against yellow fever viraemia.[47] Remarkably, the BCG vaccination of healthy volunteers has shown to induce increased levels of cytokine production upon *ex vivo* stimulation of monocytes, as well as NK cells with *M. tuberculosis* lysates, heat-killed *S. aureus*, and *C. albicans*. The enhanced function of circulating monocytes and NK cells persisted for at least 3 months after vaccination.[46,59] It is noteworthy that BCG can trigger an antigen-independent immune response known as heterologous immunity. This process is characterised by the induction of cytokines produced by T lymphocytes, such as IFN-γ and IL-17, in response to an unrelated pathogen.[60]

Furthermore, *in vitro* and *in vivo* studies have shown that the non-specific effects of BCG in bladder cancer are dependent on autophagy, in which the presence of single nucleotide polymorphisms (SNPs) in autophagy genes (*ATG2B* and *ATG5*) as well as its pharmacologic inhibition interfered with the cytokine production capacity of monocytes.[56] Besides autophagy, increased TNFα and IL-6 production by human macrophages after BCG training was associated with induction of IL-1β and polymorphism in IL-1 family genes, encoding IL-1 and IL-18 receptors.[47] In addition the inflammasome component PYCACR/ASC showed a strong impact on the production of these cytokines.[47] In line with this, the study of the effect of a genetic variation in *IL1B* and *IL32* genes led to the findings that both cytokines are associated with β-glucan-induced trained immunity.[33] Administration of β-glucan in mice induced the expansion of myeloid lineage progenitors which was associated with the induction of IL-1β and GM-CSF as well as adaptations in glucose metabolism and cholesterol biosynthesis.[61]

In addition to increased cytokine production, the functional modulation of monocytes and macrophages during trained immunity is associated with expression of Pattern Recognition Receptors (PRRs), such as TLR2, TLR4, mannose receptor C-type 1 (MRC1) and CD163 in β-glucan-trained macrophages, and CD11b and TLR4 in BCG-trained macrophages. Moreover, β-glucan-trained monocytes also showed an increased expression of costimulatory molecules (HLA-DRB1, CD40, CD86, CD80, and CD83) and CCR1 associated with dendritic cell (DC) phenotypes.[30,46] Furthermore, healthy individuals that were vaccinated with BCG and undergo a Controlled Human Malaria Infection (CHMI), showed a percentage increase of $CD56^{dim}$ NK cells expressing CD69, an activation status marker of immune cells. In addition, CD69 expression on gamma-delta (γδ) T cells, NKT cells and alpha-beta (αβ) T cells followed a similar pattern in BCG-vaccinated individuals 7 days after challenge with *Plasmodium falciparum*. Likewise, increased expression of HLA-DR and CD86 was observed in classical monocytes ($CD14^{+}CD16^{-}$) of BCG-vaccinated individuals.[48] Together, these studies indicated that increased expression of PRRs as well as costimulatory molecules and activation markers in different innate immune cell subsets are crucial for enhanced induction of host defence mechanisms.

Interestingly, it was shown that BCG mycobacterial RNA induces IL-10 production in

macrophages via TLR3-mediated activation of PI3K/AKT, indicating the capacity of BCG in modulating the immune response in addition to stimulatory effects.[62] TLR3 activation plays a crucial role in the induction of innate immune response against viruses. It was demonstrated that TLR3 agonists (Poly(I:C)) and BCG showed a synergistic effect by increasing the production of pro-inflammatory cytokines and nitric oxide (NO). Moreover, this mechanism was shown to be dependent on an auto-/paracrine-type I interferon (IFNα and IFNβ) feedback loop.[63]

Epigenetic regulation is another important mechanism that mediates trained immunity. Mechanistic investigations showed that the accumulation of epigenetic marks on several immune response genes and enhancer elements contributes to increased gene transcription. For example, changes in histone lysine methyl and acetyl modifications [H3 histones mono- or tri-methylated at lysine 4 (H3K4me1 and H3K4me3) and H3 acetylation at lysine 27 (H3K27ac)] underlie both BCG- and β-glucan-induced trained immunity. The increased cytokine production of trained macrophages is a result of higher mRNA expression, which is driven by an increased H3K4me3 at the *TNFA* and *IL6* promoters.[30,46,47] In line with this, H3K4 monomethylation (H3K4me1) enrichment is necessary for the increased responsiveness observed in monocytes challenged with β-glucan.[64]

Recently, a novel class of long non-coding RNA (lncRNA) called Immune Priming LncRNAs (IPLs) was described to regulate the epigenetic activation of trained immune genes by favoring the accumulation of H3K4me3 at its promoter regions. Mechanistic studies showed that an IPL named UMLILO (upstream master lncRNA of the inflammatory chemokine locus) could direct the WD repeat-containing protein 5 (WDR5)-mixed lineage leukaemia protein 1 complex 1 (MLL1) across the chemokine promoters (*IL8*, *CXCL1*, *CXCL2* and *CXCL3*), facilitating their H3K4me3 epigenetic priming. Furthermore, it was observed that β-glucan epigenetically reprograms immune genes by upregulating IPLs.[65] This demonstrates the important role of IPLs expression in persistent epigenetic modifications and in establishing long-term innate immune memory.

Despite functional and epigenetic reprogramming, the induction of trained immunity has been shown to depend on metabolic rewiring. It has been indicated that trained immunity induced by β-glucan in human monocytes is dependent on glucose metabolism (glycolysis), glutaminolysis and the cholesterol synthesis pathways. Inhibition of enzymes involved in these pathways prevented the epigenetic priming of genes that code for pro-inflammatory cytokines.[31,66] Of note, it has been shown that the induction of trained immunity through BCG in monocytes is associated with an increase in glycolysis and, albeit to a lesser extent, in glutaminolysis. The pharmacological modulation of glycolysis enzymes inhibits BCG-induced trained immunity by affecting the accumulation of histones marks at the *TNFA* and *IL6* promoters.[67] The metabolic alterations of different pathways in trained cells lead to the production and accumulation of various intermediary metabolites, which serve as substrates and co-factors for the activity of chromatin writers and erasers such as histone methyltransferases or acetyltransferases and demethylases or deacetylases, respectively.[68] In line with this, it has been shown that the Set7 methyltransferase (an epigenetic regulator of histone modifications) controls metabolic plasticity in oxidative phosphorylation necessary for trained immunity induced by β-glucan. Taken together, these findings demonstrate a strong correlative link between cellular metabolism and the epigenetic regulation of gene transcription, which are crucial mechanisms for the induction of trained immunity.

Considering the long-term non-specific effects exerted by the BCG vaccine and the fact that monocytes are short-lived within the circulation, recent studies aimed to determine whether trained immunity features were present in bone marrow progenitors cells. Using a mouse model, Kaufmann *et al*. discovered that BCG changes the transcriptional landscape of haematopoietic stem and progenitor cells (HSPCs), leading to cell expansion and enhanced myelopoiesis. Noteworthy, BCG-induced HSPCs produce epigenetically modified macrophages, which provide better protection against *M. tuberculosis* infection.[69] Furthermore, it has been shown that the intracellular IL-32 expression is crucial in determining the gene transcription profile in bone marrow-derived HSPCs and granulocyte macrophages progenitors cells (GMP) after BCG vaccination of human volunteers.[33] As trained immunity can occur at the progenitor cells level in the bone marrow, this creates a source of long-

lived immunologically trained cells. These transmit their phenotype to their mature cells in the periphery and establish a long-lasting trained immune response. Figure 1 illustrates the molecular mechanisms involved in the non-specific protection effects of trained immunity induced by β-glucan and the BCG vaccine.

RECENT FINDINGS ON THE USE OF THE TRAINED IMMUNITY-BASED VACCINE (BCG VACCINE) AGAINST COVID-19

BCG vaccine has a very safe track record in comparison to other vaccines. Several studies in countries with a rigorous BCG vaccination program have shown a significant correlation between BCG vaccination and decreasing COVID-19 morbidity and mortality.[22–24,70–73] A recent retrospective cohort study confirmed that BCG vaccination is safe, not associated with hyperinflammation and may reduce sickness or extreme fatigue in COVID-19 patients.[73] Routine infant BCG vaccination showed a significant impact on the prevention of local COVID-19 spread among the young generation in Japan.[74] A recent study in Israel in which individuals closely related in age, who did or did not receive BCG vaccination failed to report differences in COVID-19 prevalence.[75] This may suggest that BCG vaccination at birth would not be protective decades later. This is in line with the relatively limited duration of trained immunity induction post BCG administration.[60]

However, the studies are population-based studies and – similar to other ecological studies – are prone to be significantly biased due to several important confounding factors that can affect the

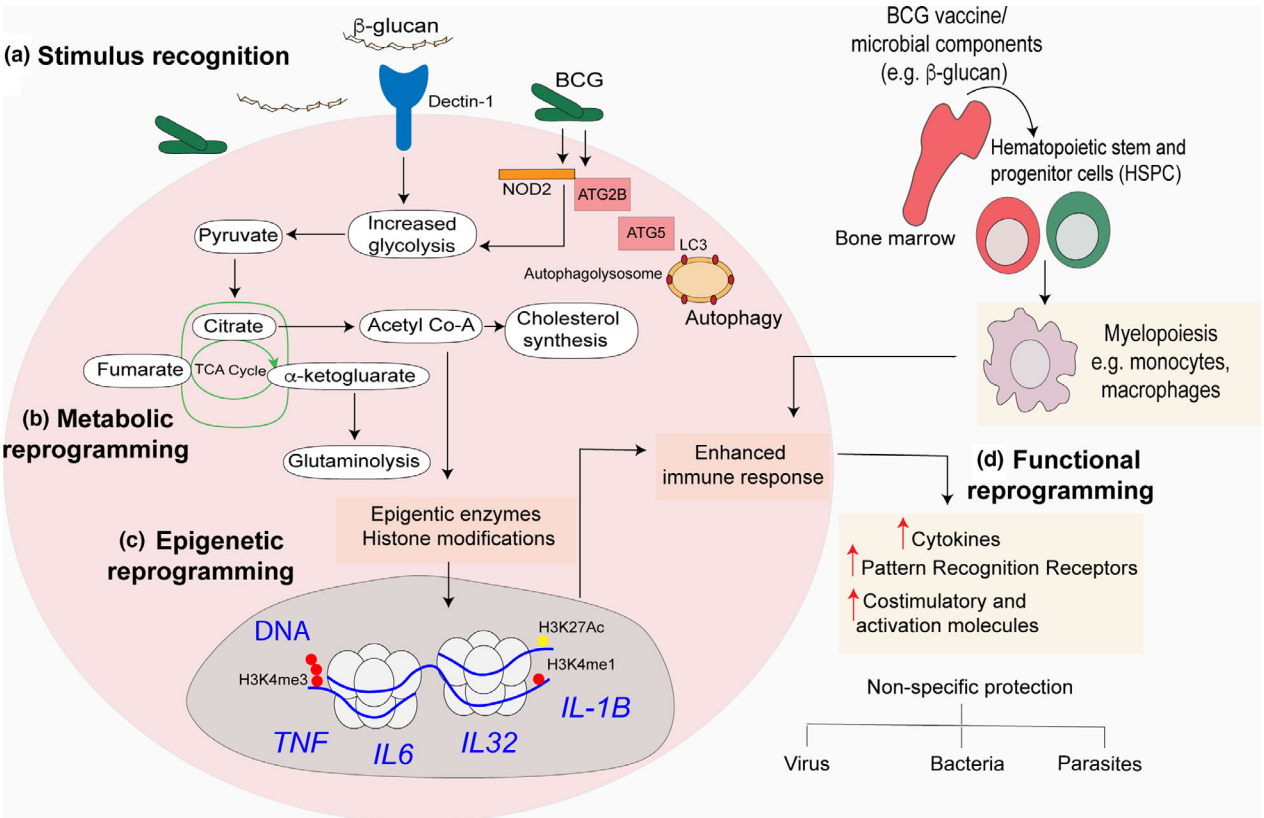

Figure 1. The molecular basis of trained immunity. The induction of trained immunity by microbial components (β-glucan) and human vaccines (BCG) involves a complex network of metabolic and epigenetic rewiring of haematopoietic stem and progenitor cells as well as circulating peripheral myeloid cells. **(a)** The process is initiated by the recognition of the stimulus by its associated pattern recognition receptors (PRRs). **(b)** Subsequently, the activation of different metabolic routes in the innate immune cells plays a central role by providing enzymes that are crucial co-factors or inhibitors of epigenetic regulators (histone modifications). **(c)** Epigenetic rewiring leads to increased gene transcription of mediators that are important for an enhanced immune response against pathogens. **(d)** The induction of such mechanisms results in increased cytokine production, PRRs expression as well as costimulatory and activation molecules upon re-stimulation. Altogether, these changes contribute to the non-specific protection against viral, bacterial and parasitic infections.

outcomes such as the distribution of ages in populations, genetic background, geographical regions, diagnostic testing rates, type of data collection, control regulations, and even public attitudes, social behaviours and obedience towards authorities. The available data could only be considered as indirect evidence of the positive effects of BCG on the COVID-19 burden.

WHEN TRAINED CELLS ENCOUNTER SARS-COV-2

Innate immune memory is established in circulating, local or tissue-resident cells as well as in progenitor stem cells through epigenetic reprogramming. We can hypothesise that when trained airway epithelial cells would be exposed to SARS-CoV2, they would produce IL-8 and recruit neutrophils towards the infected epithelia, which would then lead to efficient elimination of infected cells. Circulating monocytes would be recruited to the lung in response to MCP1 and GM-CSF. Since innate immune memory also occurs at the progenitor stem cell level, GM-CSF and G-CSF promote differentiation of innate immune cells and extend myelopoiesis, which in turn increases the number of cells migrating to the infection site, thus boosting immune response and promoting efficient local activation of the innate immune system.[3,15] TLR3 activation is induced by SARS-CoV. The BCG-trained monocytes can also produce IL10 through TLR3 activation.[62] Reactivation of this pathway by the virus can result in modulation of local inflammation.

Altogether, the dysregulation of immune responses, especially within innate immune cells, and a weak innate immune response at the site of infection, results in systemic inflammation, cytokine storm, mass virus replication, highly infectious patients and severe forms of COVID-19 (Figures 2a and 3a, b). Training of immune and non-immune cells can lead to efficient local innate immunity and elimination of the virus before it causes disease or spreads to others (Figures 2b and 3a, c). However, patients suffering from immune-mediated inflammatory diseases, HIV patients or people on immunosuppressive medication after solid organ transplantation are at much higher risk for severe adverse events from live vaccines including BCG. That is why they must be excluded from BCG vaccination program otherwise they may develop severe symptoms if they encounter COVID-19 (Figure 2c).

DURATION OF INNATE IMMUNE MEMORY

An ultimate goal in vaccine development is long-term protection. However, the unspecific effect of a vaccine cannot last very long and can be modulated by exposure to other vaccines or substances.[76] The duration of innate immune memory, induced by BCG or other vaccines and compounds, is unclear in terms of longevity. The Protective effect of BCG vaccine against *M. tuberculosis* (Mtb) decreases year by year. Based on the available data, making an accurate time estimate for BCG efficacy is very difficult. Data show that efficient protective effects of the BCG vaccine vary from a few years to several decades in different populations. It is believed that the protective efficacy of childhood BCG against all forms of TB may last for 20 years or even longer,[77,78] but that the non-specific protection is likely much shorter. The duration of innate immune memory in mice lasts for at least 3 months,[59] whereas epidemiological data showed that unspecific protective effects might last 3–5 years in human.[79] This indicates that the trained immunity approach is not a lifelong option and could only serve as a temporary solution in decreasing COVID-19 morbidity and mortality. Thus, development of a specific, well-defined vaccine remains to be considered.

CHALLENGES OF USING BCG VACCINE AGAINST COVID-19

BCG vaccine or any other compounds that are able to induce innate immune memory face several challenges when they are used on a massive scale. Since several clinical trial studies are being performed on the effect of BCG in preventing or reducing susceptibility to COVID-19, we propose the possible challenges or issues, which should be considered before, or while, BCG is being administered (Table 2).

EFFICACY OF BCG

BCG was isolated in the early 1900s, and since then the original strain has not been cloned or preserved but was merely sub-cultured in different institutes. It has been demonstrated that there are significant strain-dependent variations in microbiological and immunological properties.[80] Depending on the ability of a particular strain in

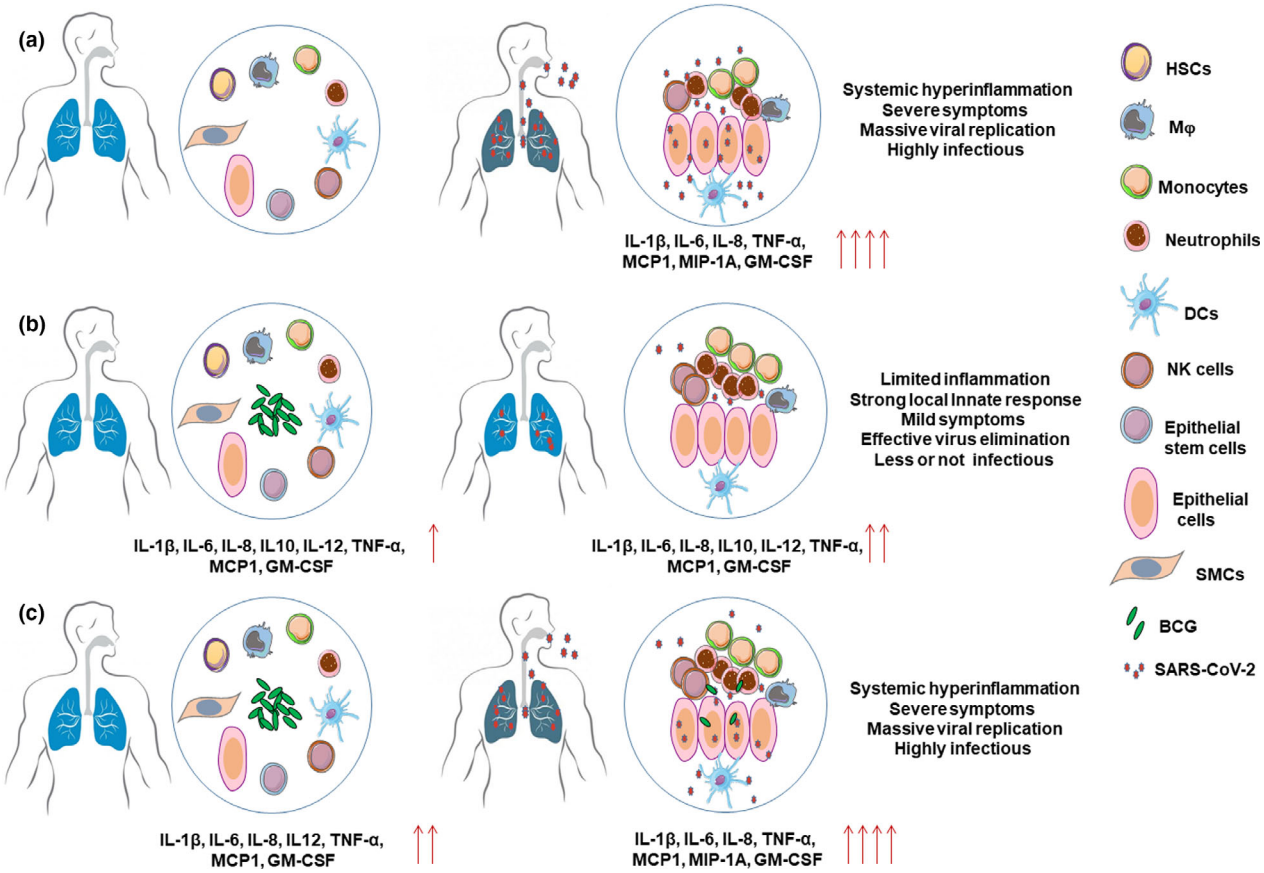

Figure 2. Possible impact of BCG vaccination on improvement of host defence against SARS-CoV-2. People with a weak immune systems or pre-existing medical conditions are at higher risk of developing sever symptoms characterised by systemic inflammation, multiple organ dysfunction and massive viral load **(a)**. In contrast, patients with BCG vaccination history develop a strong local immune response against the virus which results in a mild inflammation, less severe symptoms and an effective virus elimination **(b)**. BCG vaccination of people with weakened/ immunocompromised immune system may develop severe adverse reactions. If these patients experience COVID-19, they may develop a more severe form of the disease **(c)**.

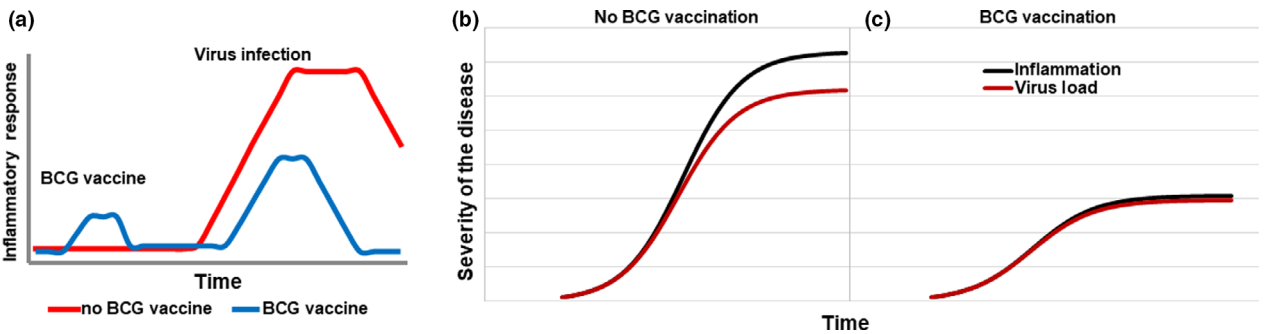

Figure 3. BCG vaccination improves immune response in COVID-19 patients. Based on current knowledge, we assume that COVID-19 patients often suffer from super inflammation and high virus load in their lung **(a, b)** and prior BCG immunisation can reduce systemic inflammation and virus load in the lung **(a–c)**.

inducing inflammatory cytokines and cytotoxic T cells in animal models, it is classified as a strong or weak strain. However, strong strains are associated with more adverse reactions.[80]

Randomised controlled trials are being performed in several countries to examine whether BCG Denmark is able to reduce the infectious rate or severity of COVID-19, or if

Table 2. Factors that influence BCG vaccination against COVID-19

Technical challenges BCG vaccine	Impact on vaccination against COVID-19
Efficacy	The most efficacious strain should be selected
Dose	The highest dose with lowest adverse effect
Vaccine shortage	Should be considered as worldwide problem
Manufacturing	Unified guideline should be prepared for producing the vaccine under GMP
Administration route	The most efficient route which induces the strongest protection has to be introduced
Underlying medical conditions	
Chronic pulmonary disease (lung fibrosis)	Exclusion factor
COPD	Beneficial effect
Asthma	Beneficial effect
Diabetes	Beneficial effect in DB type 1, DB type 1 not clear
Hypertension	Beneficial effect in mice
Chronic kidney failure	Not clear
Cardiovascular disease	Not clear
Cerebrovascular disease	Not clear
Hepatitis or liver cirrhosis	Not clear
Immunosuppressed or underlying immune deficiency	Exclusion factor
Obesity	Beneficial effect in mice
Chronic kidney disease	Safe except having an underlying immune deficiency

perhaps a different strain is a better candidate in developing efficient unspecific protection.[81] Due to a large number of people receiving BCG, making small changes and improvements will translate into a significant difference in results. Therefore, it is a crucial task to identify which BCG strain and manufacturer can develop the most efficient immunity.

PRODUCTION AND MANUFACTURING

The technology of BCG vaccine production is old. There are many small BCG vaccine suppliers in different countries, although most BCG is produced by the Serum Institute of India. Due to this, BCG vaccine production faces additional hurdle, in particular GMP issues, quality, outdated products and product licensing,[82] which may cause batch variability. Any modification in manufacturing can lead to the development of adverse events; it is necessary to have unified and defined manufacturing policies.

DOSE OF VACCINATION

The highest dose that results in the most effective immune response with minimum risk of adverse complications is the efficient dose. It was shown that a lower dose induces milder adverse effects.[83] The effective dose is dependent on BCG vaccine species and differences in manufacturing also induce a different degree of immunisation.

SHORTAGE AND AVAILABILITY OF THE VACCINE

Currently, there is a routine universal neonatal vaccination policy in 152 countries. BCG coverage was estimated to be 92% at 3 years of age.[84] Global demand for BCG vaccine was about 350 million doses in 2017.[82] BCG shortages have occurred several times, particularly in African countries.[82] Since the COVID-19 pandemic, there has been increasing concern about difficulties related to the availability of vaccines, especially in low and middle-income countries. If BCG is considered as a possibility of preventing COVID-19, the vaccine shortage will escalate and should be managed in an efficient manner. Using BCG to prevent COVID-19 must not interfere with the routine infant vaccination program.

ADMINISTRATION ROUTE

The impact of the administration route has been studied extensively. BCG vaccine was initially given orally; however, it is now injected intradermally in most countries. It was shown that immunogenicity of oral and/or intradermal administration results in distinct immune responses in human.[85] Experimental data on non-human primates showed that intranasal or endobronchial administration induces a much more effective protection than any other route.[86,87] Mukherjee *et al*. showed that that

pulmonary delivery of BCG, protected mice against lethal influenza A virus pneumonia whereas intranasal immunisation did not protect against experimental influenza (H7N9) infection, indicating that the route of BCG administration may be important.[55,88] Recently, it was shown that intravenous administration of BCG protected nine out of ten non-human primates (*Macaca mulatta*) against *M. tuberculosis* (Mtb).[89]

The natural route for SARS-CoV-2 virus infection is through the respiratory tract. Pulmonary mucosal immunisation was shown to be more efficient than intradermal administration against tuberculosis.[87] In the case of COVID-19, intranasal or pulmonary vaccination could be advantageous in developing trained immunity in the cells of the respiratory tract. Efficient training of epithelial cells in healthy individuals may trigger their immune response to effectively curb COVID-19.

POSSIBLE INFLUENCE OF BCG VACCINATION ON PATIENTS WITH PRE-EXISTING RISK FACTORS FOR COVID-19

While considering the use of BCG vaccine as a preventive approach against COVID-19, the possible impact on COVID-19 risk factors should be specifically addressed. Since SARS-CoV-2 uses ACE to enter the cells, high levels of ACE are considered to be connected to a worse outcome. Hence, the possible impact of BCG on ACE should be analysed. Current literature cannot provide data on this question. However, there are two studies from the 1980s which could not detect an elevation of ACE levels in rabbit serum[90] or guinea-pig lymph nodes[91] after BCG injection.

During the course of the ongoing pandemic, it has become clear that the presence of several risk factors may pose a life-threatening risk for COVID-19 patients (Table 2). Patients suffering from Diabetes mellitus (DM) type 1 or 2 are known to be at risk to develop prolonged and severe infections. In line with this, several studies revealed that DM patients are more susceptible to severe COVID-19.[92] Therefore, it is worth contemplating the impact of BCG on patients with DB. Of note, it was recently reported, that BCG application in patients with advanced type 1 DM led to a sustained lowering of blood glucose levels.[93,94] BCG vaccination in type 2 DM mice not only lead to decreased mortality related to tuberculosis infection, but also decreased mortality related to DM in those mice without an infection.[95] Interestingly, these diabetes-related BCG-studies showed that repeated BCG vaccination restores pancreatic islet regeneration and induces an innate immune response, which may be beneficial in treating diabetes.[93,96] Taken together, available studies support the potential use of BCG in type 1 DM patients, even in multiple doses. Admittedly, data on type 2 DM are rare, and glucose levels in case of BCG administration in DM patients should always be closely monitored.

In addition, there is evidence that patients with pre-existing cardiovascular conditions such as hypertension and coronary heart disease are more frequently become critically ill with COVID-19.[3] Hence, it is important to look at the potential consequences of BCG vaccination on atherosclerosis and hypertension. An older study reported a preventive effect of BCG against hypertension in albino rats.[97] For atherosclerosis, it is not clear whether BCG has a pro- or anti-atherosclerotic effect. On the one hand, the concept of trained immunity, in general, leads to the hypothesis that this mechanism could maintain the inflammatory process of atherogenesis.[32] Lamb *et al*.[98] reported enhanced aortic atherosclerosis in rabbits after BCG vaccination due to enhanced recruitment of peripheral monocytes towards aortic endothelium or through enhanced titres of anti-heat shock protein-60 in the sense of molecular mimicry. On the other hand, there are studies indicating an anti-atherosclerotic BCG-effect in mouse models, for example by lowering the non-HDL-cholesterol levels,[99] or by provoking the enhanced release of IL-10 instead of pro-inflammatory cytokines and via enhanced production of regulatory T cells.[100] In conclusion, it is unclear as to whether or not BCG vaccination is beneficial to patients with cardiovascular complications. However, based on the current literature, there is no evidence for harmful short term effects of a BCG vaccination in patients with existing cardiovascular disease.

Moreover, since COVID-19 is mainly a life-threatening disease due to pulmonary infection and specifically due to the development of acute severe respiratory syndrome, the possible impact of BCG on pulmonary diseases needs to be considered.

Bronchial asthma is reported to be a risk factor for worse outcomes after SARS-CoV-2 infection.[101] Clinical data indicate an inverse association

between BCG-vaccinated adults and the appearance of bronchial asthma. A randomised controlled trial in asthmatic patients showed that BCG treatment improves lung function and reduces the need for medication, possibly due to suppressing Th2-type immune responses.[102,103] A review by El-Zein *et al*.[104] discussing 23 studies concluded that the BCG vaccine in early life can protect against the development of bronchial asthma. A study on asthmatic patients revealed improved lung function after a second or third BCG vaccination.[105] Indeed, BCG re-vaccination did not exacerbate established asthma disease and in some studies even improved lung function. However, the non-specific beneficial effect of BCG vaccination on childhood asthma seems to be transient.[106] The presumption has been made that BCG could aggravate lung fibrosis by provoking augmented proliferation of pulmonary fibroblasts.[107] Thus, patients with existing pulmonary fibrosis should undergo an individual risk assessment before BCG vaccination.

POSSIBLE INCLUSION AND EXCLUSION CRITERIA FOR BCG VACCINATION – A RISK ASSESSMENT

After illuminating the potential beneficial impact of BCG vaccination on the specific risk factors for the life-threatening course of COVID-19, a detailed focus on the possible patient target groups who could receive BCG vaccination is necessary.

Attention should be paid to the conceivable adverse events of BCG vaccination to include and exclude fitting population groups. First of all, minor adverse events are well known through the worldwide BCG application over the past decades. Administering the vaccination in children is labelled as safe. Moreover, studies have proven that the usage of BCG in adults is also safe, well-tolerated and in regard to the immune reaction comparable to an early BCG vaccination.[108] Although the BCG vaccine is considered to be very safe, it may induce various complications. Based on the degree of severity, these complications are grouped into two categories. Approximately 1 in 1000 individuals develops mild complications, which are often considered as normal reactions to the vaccine including swelling, local eczema, localised abscess, formation lesion, hyperaemia or soreness formation at the site of injection.[109] 0.2–2% of BCG vaccinations cause specific local complications such as a persisting scar, limited ulcerations, subcutaneous abscesses or local lymphadenopathy.[110] Occasionally, local adverse events are due to a wrong administration of BCG, which should be injected intradermally, the procedure should always be carried out by trained personnel. However, systemic adverse reactions are rare and can include osteitis, osteomyelitis, lymphadenitis, disseminated BCG infection and in about 1–2 per million cases, lupus vulgaris can occur.[109]

Since BCG is a live vaccine, patients with pre-existing immunodeficiency are at higher risk of developing the potentially life-threatening BCG disseminated disease, which could lead to multiple organ failure through a systemic disease comparable with disseminated tuberculosis.[111] In line with that, pre-infection with HIV dramatically increases the risk of BCG disseminated disease.[112] In general, patients suffering from immune-mediated inflammatory diseases, primary immunodeficiency diseases (PIDs) such as SCID or on immunosuppressive medication after solid organ transplantation are at much higher risk for severe adverse events from live vaccines.[113] The presence of such clinical conditions should be considered as exclusion criteria (Figure 2c).

Taken together, the severity of side effects following BCG vaccination vary depending on the strain of BCG, the dose of vaccine, age or immunological and health status of patients.[109,110] Typical reactions to vaccination occur most frequently and very often are self-limiting.

Very limited data are available on the frequency of adverse effects after the first or second dose of BCG: It seems that adverse reactions are more frequent post-second dose than after the first dose.[114]

One of the biggest challenges in BCG re-vaccination is a strong local reaction due to Koch's phenomenon. This is why, before administrating a BCG vaccine, a clear and defined measure is required to limit the risks for adverse reactions such as a diagnostic test for tuberculosis infection in risk populations. Based on the literature, real Koch's phenomena are overestimated because usual adverse events are often mistaken for Koch's phenomenon.[115] However, in the majority of cases the extent of a possible skin wound was not severe enough to stop re-vaccination.[116] Indeed, a systematic follow-up with regard to adverse events should be established for BCG re-vaccination.

As mentioned earlier, SARS-CoV can enter the host cells via CD32. It has been reported that BCG vaccination increases CD32 and serum CRP level which is a major biological ligand for CD32.[117] Although more evidence is needed to confirm the significant role of CD32 and CRP, these data should be considered during BCG re-vaccination in people with the risk of immediate potential infection with SARS-CoV2. Elevation of CRP and CD32 levels is transient and BCG-vaccinated individuals could be quarantined for some time to prevent the exposure to SARS-CoV-2.

In summary, a preventive BCG vaccine should not be generally administered to patients with any form of a compromised immune system. In these cases, a substantiated risk-benefit analysis is crucial. Additionally, general vaccination rules should be followed, such as avoiding life-attenuated vaccine during an ongoing infection. Individuals should therefore be tested for a current SARS-CoV-2-infection in addition to routine investigations such as fever measurement before vaccinating with BCG.

CONCLUSIONS AND PERSPECTIVE

The COVID-19 pandemic is an extraordinary challenge for society. Old people and those with pre-existing medical conditions are more likely to experience worse symptoms and require hospital care if they have COVID-19. Large increases hospitalised cases have put health systems under immense pressure beyond their capacity particularly in low- and middle-income countries. The pandemic has drawn considerable attention from different scientific fields and brought various efforts and expertise together in order to prevent, treat or reduce the mortality. Currently, there are several different types of vaccines in development, which primarily based on spike proteins and are under various stages of clinical trials. Due to the very recent reports about SARS-CoV-2 mutations, it is predicted that the subsequent release of an effective vaccine may take longer than expected. Considering the very high transmission and mortality rate of COVID-19, even transient protection against the infection would be very beneficial. Therefore, it is important to find an alternative therapeutic approach to bridge the period until a specific vaccine is available.

Induction of trained immunity could potentially improve antiviral-host defence and better equip COVID-19 patients to fight the disease. This could be mitigation strategy to protect vulnerable people at high risk and healthcare workers and will reduce pressure on health systems and prevent hospitals from being overwhelmed by COVID-19 patients. In general, using the trained immunity approach against COVID-19 may be useful to reduce dissemination of the virus and the mortality rates. However, BCG vaccination should not be applied until the results of the ongoing randomised clinical trials have been published and BCG has been proven to be effective. Only then, effective BCG vaccination approach could potentially protect the population and reduce COVID-19 mortality and morbidity until a long-term solution in the form of a specific COVID-19 vaccine or treatment is developed.

ACKNOWLEDGMENTS

This work was supported by the Deanery of the Medical Faculty, Westfälische Wilhelms-University Münster. We also acknowledge support from the Open Access Publication Fund of the University of Münster.

CONFLICT OF INTEREST

MG Netea and LAB Joosten are scientific founders of Trained Therapeutics and Discoveries (TTxD). The other authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Yahya Sohrabi: Writing-original draft; Writing-review & editing. **Jéssica Cristina dos Santos :** Writing-original draft. **Marc Dorenkamp:** Writing-original draft. **Hannes M Findeisen:** Writing-original draft. **Rinesh Godfrey:** Writing-original draft. **Mihai G Netea:** Writing-review & editing. **Leo AB Joosten:** Supervision; Writing-review & editing.

REFERENCES

1. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. *Lancet* 2020; **395**: 470–473.
2. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. *Nat Med* 2020; **26**: 450–452.
3. Huang C, Wang Y, Li X *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497–506.
4. Liu K, Chen Y, Lin R, Han K. Clinical features of COVID-19 in elderly patients: a comparison with young and middle-aged patients. *J Infect* 2020; **80**: e14–e18.
5. Verity R, Okell LC, Dorigatti I *et al*. Estimates of the severity of coronavirus disease 2019: a model-based analysis. *Lancet Infect Dis* 2020; **20**: 669–677.

6. Kai H, Kai M. Interactions of coronaviruses with ACE2, angiotensin II, and RAS inhibitors-lessons from available evidence and insights into COVID-19. *Hypertens Res* 2020; **43**: 648–654.
7. Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: a review. *Clin Immunol* 2020; **215**: 108427.
8. Sluimer JC, Gasc JM, Hamming I *et al*. Angiotensin-converting enzyme 2 (ACE2) expression and activity in human carotid atherosclerotic lesions. *J Pathol* 2008; **215**: 273–279.
9. Sahu BR, Kampa RK, Padhi A, Panda AK. C-reactive protein: a promising biomarker for poor prognosis in COVID-19 infection. *Clin Chim Acta* 2020; **509**: 91–94.
10. Qiu F, Ma X, Shin YH *et al*. Pathogenic role of human C-reactive protein in diabetic retinopathy. *Clin Sci (Lond)* 2020; **134**: 1613–1629.
11. Anania JC, Chenoweth AM, Wines BD, Hogarth PM. The human FcγRII (CD32) family of leukocyte FcR in health and disease. *Front Immunol* 2019; **10**: 464.
12. Devaraj S, Chen X, Adams-Huet B, Jialal I. Increased expression of Fc-γ receptors on monocytes in patients with nascent metabolic syndrome. *J Clin Endocrinol Metab* 2013; **98**: E1510–E1515.
13. Tanigaki K, Sundgren N, Khera A, Vongpatanasin W, Mineo C, Shaul PW. Fcγ receptors and ligands and cardiovascular disease. *Circ Res* 2015; **116**: 368–384.
14. Walls AC, Xiong X, Park YJ *et al*. Unexpected receptor functional mimicry elucidates activation of coronavirus fusion. *Cell* 2019; **176**: 1026–1039 e1015.
15. Zhou Y, Fu B, Zheng X *et al*. Pathogenic T cells and inflammatory monocytes incite inflammatory storm in severe COVID-19 patients. *Natl Sci Rev* 2020; **7**: 998–1002. nwaa041.
16. Croxford AL, Lanzinger M, Hartmann FJ *et al*. The cytokine GM-CSF drives the inflammatory signature of $CCR2^{+}$ monocytes and licenses autoimmunity. *Immunity* 2015; **43**: 502–514.
17. Guillot L, Le Goffic R, Bloch S *et al*. Involvement of toll-like receptor 3 in the immune response of lung epithelial cells to double-stranded RNA and influenza A virus. *J Biol Chem* 2005; **280**: 5571–5580.
18. Totura AL, Whitmore A, Agnihothram S *et al*. Toll-like receptor 3 signaling via TRIF contributes to a protective innate immune response to severe acute respiratory syndrome coronavirus infection. *MBio* 2015; **6**: e00638–e00615.
19. Wen W, Su W, Tang H *et al*. Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing. *Cell Discovery* 2020; **6**: 31.
20. Cavalli G, De Luca G, Campochiaro C *et al*. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. *Lancet Rheumatol* 2020; **2**: e325–e331.
21. Padron-Regalado E. Vaccines for SARS-CoV-2: lessons from other coronavirus strains. *Infect Dis Ther* 2020; **9**: 1–20.
22. Gursel M, Gursel I. Is global BCG vaccination-induced trained immunity relevant to the progression of SARS-CoV-2 pandemic? *Allergy* 2020; **75**: 1815–1819.
23. Escobar LE, Molina-Cruz A, Barillas-Mury C. BCG vaccine protection from severe coronavirus disease 2019 (COVID-19). *Proc Natl Acad Sci USA* 2020; **117**: 17720–17726.
24. Pereira M, Paixao E, Trajman A *et al*. The need for fast-track, high-quality and low-cost studies about the role of the BCG vaccine in the fight against COVID-19. *Respir Res* 2020; **21**: 178.
25. Milutinovic B, Kurtz J. Immune memory in invertebrates. *Semin Immunol* 2016; **28**: 328–342.
26. Netea MG, Joosten LA, Latz E *et al*. Trained immunity: a program of innate immune memory in health and disease. *Science* 2016; **352**: aaf1098.
27. Schnack L, Sohrabi Y, Lagache SMM *et al*. Mechanisms of trained innate immunity in oxLDL primed human coronary smooth muscle cells. *Front Immunol* 2019; **10**: 13.
28. Naik S, Larsen SB, Gomez NC *et al*. Inflammatory memory sensitizes skin epithelial stem cells to tissue damage. *Nature* 2017; **550**: 475–480.
29. Lu Y, Sun Y, Drummer CT *et al*. Increased acetylation of H3K14 in the genomic regions that encode trained immunity enzymes in lysophosphatidylcholine-activated human aortic endothelial cells - Novel qualification markers for chronic disease risk factors and conditional DAMPs. *Redox Biol* 2019; **24**: 101221.
30. Quintin J, Saeed S, Martens JHA *et al*. Candida albicans infection affords protection against reinfection via functional reprogramming of monocytes. *Cell Host Microbe* 2012; **12**: 223–232.
31. Arts RJ, Novakovic B, Ter Horst R *et al*. Glutaminolysis and fumarate accumulation integrate immunometabolic and epigenetic programs in trained immunity. *Cell Metab* 2016; **24**: 807–819.
32. Sohrabi Y, Godfrey R, Findeisen HM. Altered cellular metabolism drives trained immunity. *Trends Endocrinol Metab* 2018; **29**: 602–605.
33. Dos Santos JC, Barroso de Figueiredo AM, Teodoro Silva MV *et al*. β-Glucan-induced trained immunity protects against *Leishmania braziliensis* infection: a crucial role for IL-32. *Cell Rep* 2019; **28**: 2659–2672 e2656.
34. Di Luzio NR, Williams DL. Protective effect of glucan against systemic *Staphylococcus aureus* septicemia in normal and leukemic mice. *Infect Immun* 1978; **20**: 804–810.
35. Krahenbuhl JL, Sharma SD, Ferraresi RW, Remington JS. Effects of muramyl dipeptide treatment on resistance to infection with *Toxoplasma gondii* in mice. *Infect Immun* 1981; **31**: 716–722.
36. Ribes S, Meister T, Ott M *et al*. Intraperitoneal prophylaxis with CpG oligodeoxynucleotides protects neutropenic mice against intracerebral Escherichia coli K1 infection. *J Neuroinflammation* 2014; **11**: 14.
37. Bardel E, Doucet-Ladeveze R, Mathieu C, Harandi AM, Dubois B, Kaiserlian D. Intradermal immunisation using the TLR3-ligand Poly (I:C) as adjuvant induces mucosal antibody responses and protects against genital HSV-2 infection. *NPJ Vaccines* 2016; **1**: 16010.
38. Rieckmann A, Villumsen M, Sorup S *et al*. Vaccinations against smallpox and tuberculosis are associated with better long-term survival: a Danish case-cohort study 1971–2010. *Int J Epidemiol* 2017; **46**: 695–705.
39. Aaby P, Martins CL, Garly ML *et al*. Non-specific effects of standard measles vaccine at 4.5 and 9 months of age on childhood mortality: randomised controlled trial. *BMJ* 2010; **341**: c6495.

40. Biering-Sorensen S, Aaby P, Napirna BM *et al*. Small randomized trial among low-birth-weight children receiving bacillus Calmette-Guerin vaccination at first health center contact. *Pediatr Infect Dis J* 2012; **31**: 306–308.
41. Nemes E, Geldenhuys H, Rozot V *et al*. Prevention of *M. tuberculosis* Infection with H4:IC31 Vaccine or BCG Revaccination. *N Engl J Med* 2018; **379**: 138–149.
42. Biering-Sorensen S, Aaby P, Lund N *et al*. Early BCG-Denmark and neonatal mortality among infants weighing <2500 g: a randomized controlled trial. *Clin Infect Dis* 2017; **65**: 1183–1190.
43. Ohrui T, Nakayama K, Fukushima T, Chiba H, Sasaki H. [Prevention of elderly pneumonia by pneumococcal, influenza and BCG vaccinations]. *Nihon Ronen Igakkai Zasshi Japanese Journal of Geriatrics* 2005; **42**: 34–36.
44. Kolmel KF, Grange JM, Krone B *et al*. Prior immunisation of patients with malignant melanoma with vaccinia or BCG is associated with better survival. An European Organization for Research and Treatment of Cancer cohort study on 542 patients. *Eur J Cancer* 2005; **41**: 118–125.
45. Lankes HA, Fought AJ, Evens AM, Weisenburger DD, Chiu BC. Vaccination history and risk of non-Hodgkin lymphoma: a population-based, case-control study. *Cancer Causes Control* 2009; **20**: 517–523.
46. Kleinnijenhuis J, Quintin J, Preijers F *et al*. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. *Proc Natl Acad Sci USA* 2012; **109**: 17537–17542.
47. Arts RJW, Moorlag S, Novakovic B *et al*. BCG vaccination protects against experimental viral infection in humans through the induction of cytokines associated with trained immunity. *Cell Host Microbe* 2018; **23**: 89–100 e105.
48. Walk J, de Bree LCJ, Graumans W *et al*. Outcomes of controlled human malaria infection after BCG vaccination. *Nat Commun* 2019; **10**: 874.
49. Pereira LI, Dorta ML, Pereira AJ *et al*. Increase of NK cells and proinflammatory monocytes are associated with the clinical improvement of diffuse cutaneous leishmaniasis after immunochemotherapy with BCG/Leishmania antigens. *Am J Trop Med Hyg* 2009; **81**: 378–383.
50. Fortier AH, Mock BA, Meltzer MS, Nacy CA. Mycobacterium bovis BCG-induced protection against cutaneous and systemic *Leishmania major* infections of mice. *Infect Immun* 1987; **55**: 1707–1714.
51. Leentjens J, Kox M, Stokman R *et al*. BCG vaccination enhances the immunogenicity of subsequent influenza vaccination in healthy volunteers: a randomized, placebo-controlled pilot study. *J Infect Dis* 2015; **212**: 1930–1938.
52. Angelidou A, Conti MG, Diray-Arce J *et al*. Licensed Bacille Calmette-Guerin (BCG) formulations differ markedly in bacterial viability, RNA content and innate immune activation. *Vaccine* 2020; **38**: 2229–2240.
53. Aldovini A, Young RA. Humoral and cell-mediated immune responses to live recombinant BCG-HIV vaccines. *Nature* 1991; **351**: 479–482.
54. Uno-Furuta S, Matsuo K, Tamaki S *et al*. Immunization with recombinant Calmette-Guerin bacillus (BCG)-hepatitis C virus (HCV) elicits HCV-specific cytotoxic T lymphocytes in mice. *Vaccine* 2003; **21**: 3149–3156.
55. Mukherjee S, Subramaniam R, Chen H, Smith A, Keshava S, Shams H. Boosting efferocytosis in alveolar space using BCG vaccine to protect host against influenza pneumonia. *PLoS One* 2017; **12**: e0180143.
56. Buffen K, Oosting M, Quintin J *et al*. Autophagy controls BCG-induced trained immunity and the response to intravesical BCG therapy for bladder cancer. *PLoS Pathog* 2014; **10**: e1004485.
57. Barton ES, White DW, Cathelyn JS *et al*. Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature* 2007; **447**: 326–329.
58. Hong M, Sandalova E, Low D *et al*. Trained immunity in newborn infants of HBV-infected mothers. *Nat Commun* 2015; **6**: 6588.
59. Kleinnijenhuis J, Quintin J, Preijers F *et al*. BCG-induced trained immunity in NK cells: role for non-specific protection to infection. *Clin Immunol* 2014; **155**: 213–219.
60. Kleinnijenhuis J, Quintin J, Preijers F *et al*. Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. *J Innate Immun* 2014; **6**: 152–158.
61. Mitroulis I, Ruppova K, Wang B *et al*. Modulation of myelopoiesis progenitors is an integral component of trained immunity. *Cell* 2018; **172**: 147–161 e112.
62. Bai W, Liu H, Ji Q *et al*. TLR3 regulates mycobacterial RNA-induced IL-10 production through the PI3K/AKT signaling pathway. *Cell Signal* 2014; **26**: 942–950.
63. Speth MT, Repnik U, Muller E *et al*. Poly(I:C)-encapsulating nanoparticles enhance innate immune responses to the tuberculosis vaccine bacille Calmette-Guerin (BCG) via synergistic activation of innate immune receptors. *Mol Pharm* 2017; **14**: 4098–4112.
64. Novakovic B, Habibi E, Wang SY *et al*. β-Glucan reverses the epigenetic state of LPS-induced immunological tolerance. *Cell* 2016; **167**: 1354–1368 e1314.
65. Fanucchi S, Fok ET, Dalla E *et al*. Immune genes are primed for robust transcription by proximal long noncoding RNAs located in nuclear compartments. *Nat Genet* 2019; **51**: 138–150.
66. Cheng SC, Quintin J, Cramer RA *et al*. mTOR/HIF1α-mediated aerobic glycolysis as metabolic basis for trained immunity. *Science* 2014; **345**: 1250684.
67. Arts RJW, Carvalho A, La Rocca C *et al*. Immunometabolic pathways in BCG-induced trained immunity. *Cell Rep* 2016; **17**: 2562–2571.
68. Keating ST, El-Osta A. Epigenetics and metabolism. *Circ Res* 2015; **116**: 715–736.
69. Kaufmann E, Sanz J, Dunn JL *et al*. BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. *Cell* 2018; **172**: 176–190 e119.
70. Ventura L, Vitali M, Romano Spica V. BCG vaccination and socioeconomic variables vs COVID-19 global features: clearing up a controversial issue. *Allergy* 2020. https://doi.org/10.1111/all.14524. Online ahead of print.
71. Ozdemir C, Kucuksezer UC, Tamay ZU. Is BCG vaccination effecting the spread and severity of COVID-19? *Allergy* 2020; **75**: 1824–1827.
72. Weng CH, Saal A, Butt WW *et al*. Bacillus Calmette-Guerin vaccination and clinical characteristics and outcomes of COVID-19 in Rhode Island, United States: a cohort study. *Epidemiol Infect* 2020; **148**: e140.

73. Moorlag SJCFM, van Deuren RC, van Werkhoven CH *et al*. Safety and COVID-19 symptoms in individuals recently vaccinated with BCG: a retrospective cohort study. *Cell Rep Med* 2020; **1**: 100073.
74. Kinoshita M, Tanaka M. Impact of routine infant BCG vaccination in young generation on prevention of local COVID-19 spread in Japan. *J Infect* 2020; **81**: 625–633.
75. Hamiel U, Kozer E, Youngster I. SARS-CoV-2 Rates in BCG-Vaccinated and Unvaccinated Young Adults. *JAMA* 2020; **323**: 2340–2341.
76. Pollard AJ, Finn A, Curtis N. Non-specific effects of vaccines: plausible and potentially important, but implications uncertain. *Arch Dis Child* 2017; **102**: 1077–1081.
77. Barreto ML, Cunha SS, Pereira SM *et al*. Neonatal BCG protection against tuberculosis lasts for 20 years in Brazil. *Int J Tuberc Lung Dis* 2005; **9**: 1171–1173.
78. Nguipdop-Djomo P, Heldal E, Rodrigues LC, Abubakar I, Mangtani P. Duration of BCG protection against tuberculosis and change in effectiveness with time since vaccination in Norway: a retrospective population-based cohort study. *Lancet Infect Dis* 2016; **16**: 219–226.
79. Netea MG, Schlitzer A, Placek K, Joosten LAB, Schultze JL. Innate and adaptive immune memory: an evolutionary continuum in the host's response to pathogens. *Cell Host Microbe* 2019; **25**: 13–26.
80. Ritz N, Dutta B, Donath S *et al*. The influence of bacille Calmette-Guerin vaccine strain on the immune response against tuberculosis: a randomized trial. *Am J Respir Crit Care Med* 2012; **185**: 213–222.
81. Curtis N, Sparrow A, Ghebreyesus TA, Netea MG. Considering BCG vaccination to reduce the impact of COVID-19. *Lancet* 2020; **395**: 1545–1546.
82. Cernuschi T, Malvolti S, Nickels E, Friede M. Bacillus Calmette-Guerin (BCG) vaccine: a global assessment of demand and supply balance. *Vaccine* 2018; **36**: 498–506.
83. Kandeel W, Abdelal A, Elmohamady BN *et al*. A comparative study between full-dose and half-dose intravesical immune bacille Calmette-Guerin injection in the management of superficial bladder cancer. *Arab J Urol* 2015; **13**: 233–237.
84. Roy P, Vekemans J, Clark A, Sanderson C, Harris RC, White RG. Potential effect of age of BCG vaccination on global paediatric tuberculosis mortality: a modelling study. *Lancet Glob Health* 2019; **7**: e1655–e1663.
85. Hoft DF, Xia M, Zhang GL *et al*. PO and ID BCG vaccination in humans induce distinct mucosal and systemic immune responses and $CD4^+$ T cell transcriptomal molecular signatures. *Mucosal Immunol* 2018; **11**: 486–495.
86. Goonetilleke NP, McShane H, Hannan CM, Anderson RJ, Brookes RH, Hill AV. Enhanced immunogenicity and protective efficacy against Mycobacterium tuberculosis of bacille Calmette-Guerin vaccine using mucosal administration and boosting with a recombinant modified vaccinia virus Ankara. *J Immunol* 2003; **171**: 1602–1609.
87. Verreck FAW, Tchilian EZ, Vervenne RAW *et al*. Variable BCG efficacy in rhesus populations: pulmonary BCG provides protection where standard intra-dermal vaccination fails. *Tuberculosis* 2017; **104**: 46–57.
88. de Bree LCJ, Marijnissen RJ, Kel JM *et al*. Bacillus Calmette-Guerin-induced trained immunity is not protective for experimental influenza A/Anhui/1/2013 (H7N9) infection in mice. *Front Immunol* 2018; **9**: 869.
89. Darrah PA, Zeppa JJ, Maiello P *et al*. Prevention of tuberculosis in macaques after intravenous BCG immunization. *Nature* 2020; **577**: 95–102.
90. Lieberman J, Krauthammer M, Sastre A. Serum angiotensin-converting-enzyme in rabbits with and without pulmonary granulomatosis. Granulomatosis induced with complete-Freund's-adjuvant or BCG. *Sarcoidosis* 1986; **3**: 60–66.
91. Rea TH, Narayanan RB, Turk JL. Lysozyme and angiotensin converting enzyme levels in experimental mycobacterial granulomas. *J Pathol* 1983; **139**: 207–216.
92. Guo W, Li M, Dong Y, *et al*. Diabetes is a risk factor for the progression and prognosis of COVID -19. *Diabetes Metab Res Rev* 2020; e3319.
93. Chang YC, Lin CJ, Hsiao YH, Chang YH, Liu SJ, Hsu HY. Therapeutic effects of BCG vaccination on type 1 diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials. *J Diabetes Res* 2020; **2020**: 8954125.
94. Kodama S, Kuhtreiber W, Fujimura S, Dale EA, Faustman DL. Islet regeneration during the reversal of autoimmune diabetes in NOD mice. *Science* 2003; **302**: 1223–1227.
95. Radhakrishnan RK, Thandi RS, Tripathi D *et al*. BCG vaccination reduces the mortality of Mycobacterium tuberculosis-infected type 2 diabetes mellitus mice. *JCI Insight* 2020; **5**: e133788.
96. Faustman DL, Wang L, Okubo Y *et al*. Proof-of-concept, randomized, controlled clinical trial of Bacillus-Calmette-Guerin for treatment of long-term type 1 diabetes. *PLoS One* 2012; **7**: e41756.
97. Somogyi I, Rigo J, Sos J. The prevention of experimental hypertension and arteriosclerosis with B.C.G. and tuberculin. *Lancet* 1962; **2**: 280–281.
98. Lamb DJ, Ferns GA. The magnitude of the immune response to heat shock protein-65 following BCG immunisation is associated with the extent of experimental atherosclerosis. *Atherosclerosis* 2002; **165**: 231–240.
99. van Dam AD, Bekkering S, Crasborn M *et al*. BCG lowers plasma cholesterol levels and delays atherosclerotic lesion progression in mice. *Atherosclerosis* 2016; **251**: 6–14.
100. Ovchinnikova OA, Berge N, Kang C *et al*. Mycobacterium bovis BCG killed by extended freeze-drying induces an immunoregulatory profile and protects against atherosclerosis. *J Intern Med* 2014; **275**: 49–58.
101. Goyal P, Choi JJ, Pinheiro LC *et al*. Clinical characteristics of Covid-19 in New York City. *N Engl J Med* 2020; **382**: 2372–2374.
102. Choi IS, Koh YI. Therapeutic effects of BCG vaccination in adult asthmatic patients: a randomized, controlled trial. *Ann Allergy Asthma Immunol* 2002; **88**: 584–591.

103. Park SS, Heo EY, Kim DK, Chung HS, Lee CH. The association of BCG vaccination with atopy and asthma in adults. *Int J Med Sci* 2015; **12**: 668–673.
104. El-Zein M, Parent ME, Benedetti A, Rousseau MC. Does BCG vaccination protect against the development of childhood asthma? A systematic review and meta-analysis of epidemiological studies. *Int J Epidemiol* 2010; **39**: 469–486.
105. Datau EA, Mewengkang H, Matheos JC *et al*. Clinical efficacy and laboratory improvement of bacillus calmette-guerin vaccination on adult atopic asthma: a cohort study. *World Allergy Organ J* 2008; **1**: 63–69.
106. Linehan MF, Nurmatov U, Frank TL, Niven RM, Baxter DN, Sheikh A. Does BCG vaccination protect against childhood asthma? Final results from the Manchester Community Asthma Study retrospective cohort study and updated systematic review and meta-analysis. *J Allergy Clin Immunol* 2014; **133**: 688–695 e614.
107. Chyczewska E, Chyczewski L, Bankowski E, Sulkowski S, Niklinski J. Stimulation of alveolar macrophages by BCG vaccine enhances the process of lung fibrosis induced by bleomycin. *Folia Histochem Cytobiol* 1993; **31**: 113–116.
108. Hatherill M, Geldenhuys H, Pienaar B *et al*. Safety and reactogenicity of BCG revaccination with isoniazid pretreatment in TST positive adults. *Vaccine* 2014; **32**: 3982–3988.
109. Venkataraman A, Yusuff M, Liebeschuetz S, Riddell A, Prendergast AJ. Management and outcome of Bacille Calmette-Guerin vaccine adverse reactions. *Vaccine* 2015; **33**: 5470–5474.
110. Decaestecker K, Oosterlinck W. Managing the adverse events of intravesical bacillus Calmette-Guerin therapy. *Res Rep Urol* 2015; **7**: 157–163.
111. Talbot EA, Perkins MD, Silva SF, Frothingham R. Disseminated bacille Calmette-Guerin disease after vaccination: case report and review. *Clin Infect Dis* 1997; **24**: 1139–1146.
112. Hesseling AC, Marais BJ, Gie RP *et al*. The risk of disseminated Bacille Calmette-Guerin (BCG) disease in HIV-infected children. *Vaccine* 2007; **25**: 14–18.
113. Croce E, Hatz C, Jonker EF, Visser LG, Jaeger VK, Buhler S. Safety of live vaccinations on immunosuppressive therapy in patients with immune-mediated inflammatory diseases, solid organ transplantation or after bone-marrow transplantation – a systematic review of randomized trials, observational studies and case reports. *Vaccine* 2017; **35**: 1216–1226.
114. Dourado I, Rios MH, Pereira SM *et al*. Rates of adverse reactions to first and second doses of BCG vaccination: results of a large community trial in Brazilian schoolchildren. *Int J Tuberc Lung Dis* 2003; **7**: 399–402.
115. Kato S, Tokunaga O, Yoshiyama T. Analysis of Koch's phenomenon by BCg vaccination with the multi-puncture method in Japan. *Kekkaku* 2010; **85**: 777–782.
116. Ferreira AA, Ferreira Mde F, Macedo EA *et al*. [BCG revaccination in school children: evolution of the lesion at the vaccination site between 48 hours and 10 weeks]. *J Pediatr* 2002; **78**: 289–294.
117. Boer MC, Prins C, van Meijgaarden KE, van Dissel JT, Ottenhoff TH, Joosten SA. Mycobacterium bovis BCG vaccination induces divergent proinflammatory or regulatory T cell responses in adults. *Clin Vaccine Immunol* 2015; **22**: 778–788.
118. Marakalala MJ, Williams DL, Hoving JC, Engstad R, Netea MG, Brown GD. Dectin-1 plays a redundant role in the immunomodulatory activities of β-glucan-rich ligands *in vivo*. *Microbes Infect* 2013; **15**: 511–515.
119. Kumaki Y, Salazar AM, Wandersee MK, Barnard DL. Prophylactic and therapeutic intranasal administration with an immunomodulator, Hiltonol((R)) (Poly IC:LC), in a lethal SARS-CoV-infected BALB/c mouse model. *Antiviral Res* 2017; **139**: 1–12.
120. Ishihara C, Mizukoshi N, Iida J, Kato K, Yamamoto K, Azuma I. Suppression of Sendai virus growth by treatment with N^{α}-acetylmuramyl-L-alanyl-D-isoglutaminyl-N^{ε}-stearoyl-L-lysine in mice. *Vaccine* 1987; **5**: 295–301.
121. Dietrich FM, Hochkeppel HK, Lukas B. Enhancement of host resistance against virus infections by MTP-PE, a synthetic lipophilic muramyl peptide–I. Increased survival in mice and guinea pigs after single drug administration prior to infection, and the effect of MTP-PE on interferon levels in sera and lungs. *Int J Immunopharmacol* 1986; **8**: 931–942.
122. Cheng SC, Scicluna BP, Arts RJ *et al*. Broad defects in the energy metabolism of leukocytes underlie immunoparalysis in sepsis. *Nat Immunol* 2016; **17**: 406–413.
123. Floc'h F, Werner GH. Increased resistance to virus infections of mice inoculated with BCG (Bacillus calmette-guerin). *Ann Immunol* 1976; **127**: 173–186.
124. Sorup S, Benn CS, Poulsen A, Krause TG, Aaby P, Ravn H. Live vaccine against measles, mumps, and rubella and the risk of hospital admissions for nontargeted infections. *JAMA* 2014; **311**: 826–835.
125. Tielemans S, de Melker HE, Hahne SJM *et al*. Non-specific effects of measles, mumps, and rubella (MMR) vaccination in high income setting: population based cohort study in the Netherlands. *BMJ* 2017; **358**: j3862.
126. Sorup S, Benn CS, Stensballe LG, Aaby P, Ravn H. Measles-mumps-rubella vaccination and respiratory syncytial virus-associated hospital contact. *Vaccine* 2015; **33**: 237–245.
127. Sorup S, Stensballe LG, Krause TG, Aaby P, Benn CS, Ravn H. Oral polio vaccination and hospital admissions with non-polio infections in Denmark: Nationwide retrospective cohort study. *Open Forum Infect Dis* 2016; **3**: ofv204.
128. Tarancon R, Dominguez-Andres J, Uranga S *et al*. New live attenuated tuberculosis vaccine MTBVAC induces trained immunity and confers protection against experimental lethal pneumonia. *PLoS Pathog* 2020; **16**: e1008404.
129. Yao Y, Jeyanathan M, Haddadi S *et al*. Induction of autonomous memory alveolar macrophages requires T cell help and is critical to trained immunity. *Cell* 2018; **175**: 1634–1650 e1617.