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IS GLOBAL BCG VACCINATION-INDUCED TRAINED IMMUNITY RELEVANT TO THE PROGRESSION OF SARS-CoV-2 PANDEMIC?

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Letter to the Editor,

In January, WHO Director General Tedros Adhanom Ghebreyesus said his “greatest concern” was COVID-19 spreading in countries with fragile health systems. Although countries like India, Philippines,Thailand, and Nepal have reported their first confirmed cases of the SARS-CoV-2 virus in January, widespread community spread have not been reported. Contrary to such justified expectations/predictions, on March 13 2020, WHO declared Europe as the epicenter of the pandemic. Even though we are still in the midst of the coronavirus pandemic, the disproportionately smaller number of cases reported from disadvantaged/low income countries remains puzzling. We hypothesize that general BCG vaccination policies adopted by different countries might have impacted the transmission patterns and/or COVID-19 associated morbidity and mortality.

Vaccines provide protection to a particular pathogen by inducing effector mechanisms directed to that pathogen. However, certain attenuated vaccines like the Bacillus Calmette–Guerin (BCG), can also protect against unrelated pathogens, some of which cause acute respiratory tract infections[1,S1-S6]. The underlying mechanism for the BCG vaccination-induced non-specific protection is thought to be mediated via the induction of innate immune memory, or “trained immunity, as was first proposed by Netea and collaborators.[2] Trained-immunity inducing agents reprogramme bone marrow hematopietic stem cells and multipotent progenitors through epigenetic and metabolic changes, resulting in a more robust response in differentiated innate immune cells, following encounter with a pathogen[S7-S8]. Of interest, in a randomized placebo-controlled human study, BCG vaccination was demonstrated to induce epigenetic reprograming in monocytes, conferring protection against experimental infection with an attenuated yellow fever virus vaccine strain.[3]

Based on these observations, we hypothesized that countries who continue BCG immunization programs would contain the spread of this new coronavirus better than those that did not have or have ceased their national BCG vaccination programs.

To check the validity of this hypothesis, we compared the number of cases and deaths per million people from all countries with at least 500 (23 March) or 1000 cases (29

and 31 March) according to their BCG vaccination status (Figure 1A, 1B and Supplementary Figure 1 (for updated data from 6 April) and Supplementary Table 1). Cases/million in countries with a national BCG vaccination programme were statistically significantly lower than those that did not have/ceased their national BCG vaccination programs ($P<0.0001$). We also compared the number of deaths per million. Results showed that COVID-19-associated deaths relative to the size of the population were significantly lower in countries with a national BCG vaccination programme than those without BCG vaccination ($P<0.0058$ and $P<0.0001$ for 23 March and 29, 31 March, respectively). To correct for different stages of the spread of disease, we downloaded the data showing the total confirmed deaths since the 5th death from Our World in Data web site (https://ourworldindata.org/grapher/covid-confirmed-deaths-since-5th-death). Instead of the 5th death as day 0, we chose the 100th death as day 0. The total deaths on 14th or 20th days after the 100th death were divided by the population of each country to obtain deaths/million. All countries that had data on these days were included and the comparison between the BCG vaccinated and unvaccinated populations were made (Figure 1 C). Using this "disease stage normalized" data, there was still a highly significant difference between countries that adhered to national BCG vaccination policy versus those that had ceases/never had a national programme (Figure 1C). If BCG vaccination has a general non-specific protective effect against spread of SARS-CoV-2 or COVID-19-associated morbidity and mortality, then BCG re-vaccination of populations offer a viable alternative of partial protection until a specific vaccine is available. The duration of BCG-induced trained immunity or how different vaccine strains compare in terms of longevity of induced innate memory is not known. Work by Netea et al show that the "trained immunity status" is maintained for at least a year (the maximum time point they measured)[S9]. BCG-induced protection against tuberculosis lasts for approximately 20 years and wanes thereafter[S10]. If one assumes that BCG-induced non-specific protective effect also lasts for 20 years and gradually wanes, then there should be a difference between countries that have stopped BCG vaccination earlier versus later. To assess this possibility, we analysed data from 13 European Countries that have ceased their national BCG vaccination programmes (Supplementary Table 2). According to this, 5 Countries (Norway,

France, Finland, UK and Germany) had ceased vaccination in the last 2 decades, whereas 8 countries had dropped national BCG vaccination in the last 3-4 decades (Austria, Belgium, Switzerland, Denmark, Spain, Netherlands, Sweden) or had no national coverage (Italy, represented with an arbitrary value of 50). We then downloaded the data representing the daily confirmed COVID-19 deaths per million people from OUR World in Data website (https://ourworldindata.org/grapher/covid-daily-deaths-trajectory-per-million) for these 13 countries (Summarized in Supplementary Table 3). We chose the deaths/million on day 7 of the epidemic (highlighted in bold in the supplementary Table 3) as the time point to compare the deaths/million between countries (i.e. before their health infrastructure was possibly overwhelmed). Our results demonstrated a statistically significant difference in deaths/million on day 7 since the daily confirmed deaths reached 0.1/million (Mann-Whitney U test; P=0.0109) between countries that had ceased vaccination in the last 2 versus the last 3-4 decades (Figure 1D). This result suggests that BCG-vaccination induced heterologous non-specific protective effect could be of long-lasting duration (~ 20 years) and therefore could potentially impact the dynamics of SARS-CoV-2-associated community spread and/or disease severity.

There is also the question of which BCG vaccine strain to chose. The BCG vaccine strains used by different countries vary widely. BCG vaccine was first introduced in 1921 and the seeds were distributed to various countries. During their passage, BCG strains accumulated genomic alterations, including deletions, single-nucleotide polymorphisms and duplications, leading to the emergence of several substrains.[4] Based on their tandem duplication variants (DU2), BCG vaccines fall into 4 groups (Figure 2 B). The DU2-I and II group consists of “early” BCG vaccine strains, (Japan, Russia and Moreau/Brazil), whereas DU2-III-IV are considered as more distant “late” vaccine strains (like Pasteur, Denmark and Connaught).[4] The strains differ in terms of their growth characteristics, biochemistry, immunogenicity, and virulence. The late BCG strains are defective in production of cell wall methoxymycolic acids and possess only the alpha- and ketomycolic acids.[5] Consistent with this, early BCG strains persist up to 6 months in the mesenteric lymph nodes of vaccinated children, whereas no live bacteria could be detected in late strain vacinees. Similarly,

methoxymycolate- producing early strains are more potent immunostimulating agents than the late strains.[6] Mycolic acids can condition macrophages to produce higher levels of IFN-γ, myeloperoxidase and TNF-α upon renewed exposure to innate triggers.[7] Accordingly, mycolic acids constitute an important group of ligands capable of inducing trained immunity. Methoxymycolic acids are inflammatory and can activate macrophages, whereas, ketomycolic acids promote anti-inflammatory, alternatively activated macrophages.[7] Since the persistence and immunostimulatory properties of BCG strains differ, their potential to induce trained immunity in vaccinated individuals could also vary.

When we analyzed available data on BCG vaccine strains used in different countries (Figure 2 A, modified from references 8 and 9), Iran and China, emerged as local producers of their own vaccines. Evidence suggests that the BCG vaccine strain in Iran is BCG-Pasteur 1173p2[S11] and the one in China is a strain derived from Glaxo 1077[S12], representing the most modified and highly attenuated strains deficient of methoxymycolic acids when compared to the Japan and Russia strains. It is conceivable that the trained immunity induced by the Iran and China BCG vaccine strains are short-lived compared to older strains widely utilized by other countries.

The lower than expected number of cases detected in countries in Asia and Africa with extensive travel and trade links with China might stem from the BCG immunization-induced heterologous protective activity of the vaccine. Should this hypothesis hold its ground, then there would be important repercussions that could save lives. Since BCG vaccination was previously demonstrated to prevent acute respiratory tract infections even in the elderly, until a specific vaccine is developed, the results of clinical trials testing for BCG vaccine as defense against SARS-CoV-2 could be critical in the fight against the new coronavirus pandemic (list of ongoing/planned clinical trials are provided in Supplementary Table 4).

Figure Legends

Figure 1. Comparison of number of cases/million (**A**) and deaths/million (**B**) disease stage normalized deaths/million (**C**) between countries that follow a national BCG immunization programme (blue circles) and those that did not have or have ceased their national BCG vaccination programmes. (red squares) (**D**), Comparison of deaths/million between European countries that had ceased BCG vaccination in the last 2 (late) versus the last 3-4 decades (early). Statistical comparison was based on two-tailed Mann Whitney U test. Coronavirus related statistics were based on data obtained from https://www.worldometers.info/coronavirus/ (Figures 1A and 1B, according to the latest update on March 23, 29 and 31) and from Our World in Data (links of data used were provided in the main text of the manuscript) .

Figure 2. (**A)** BCG vaccine strains used Worldwide (Modified from data presented in Refererences 8 and 9). Countries that have no current national BCG vaccination programme are shown in red. Globally used BCG vaccine strains were: Russia (dark blue), locally produced strain (gray), Denmark (orange), Japan (light blue), Brazil (purple), Pasteur (yellow) and countries that used more than one strain (green). (**B)** Genealogy of BCG Vaccine Strains. Modified from Brosch et al. (2007).

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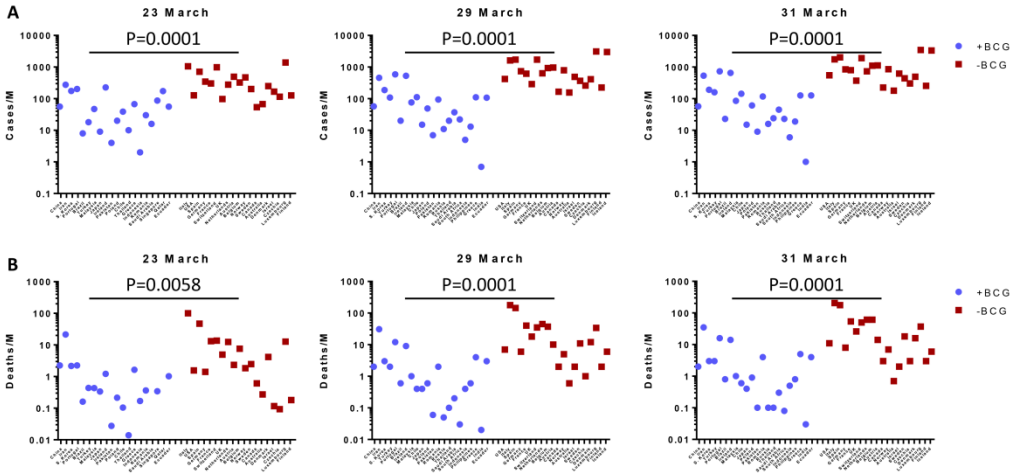

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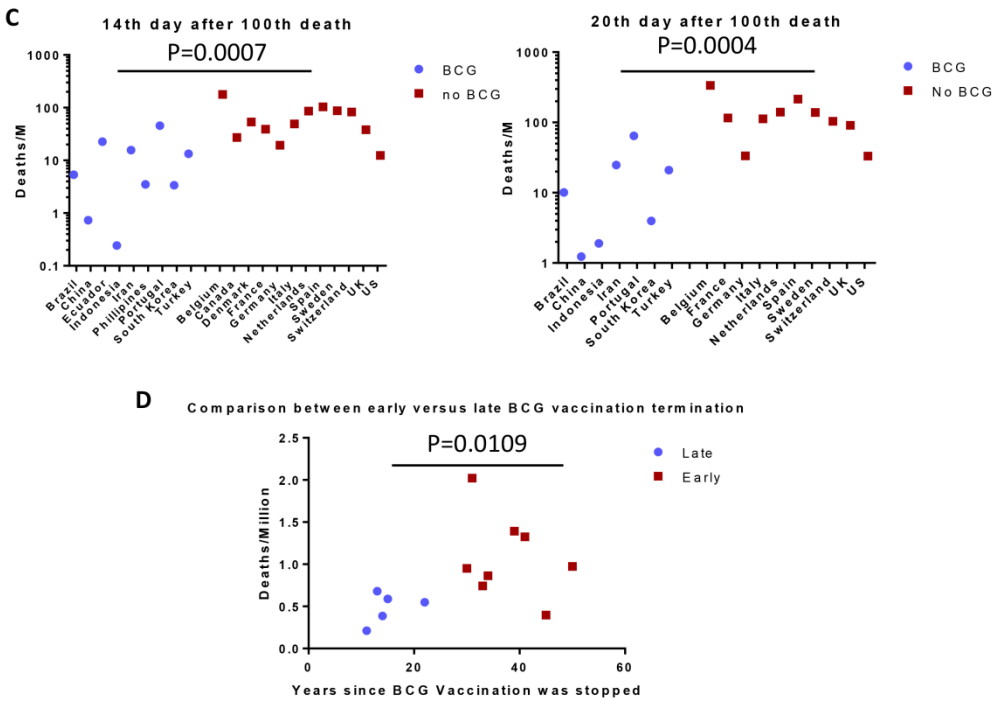

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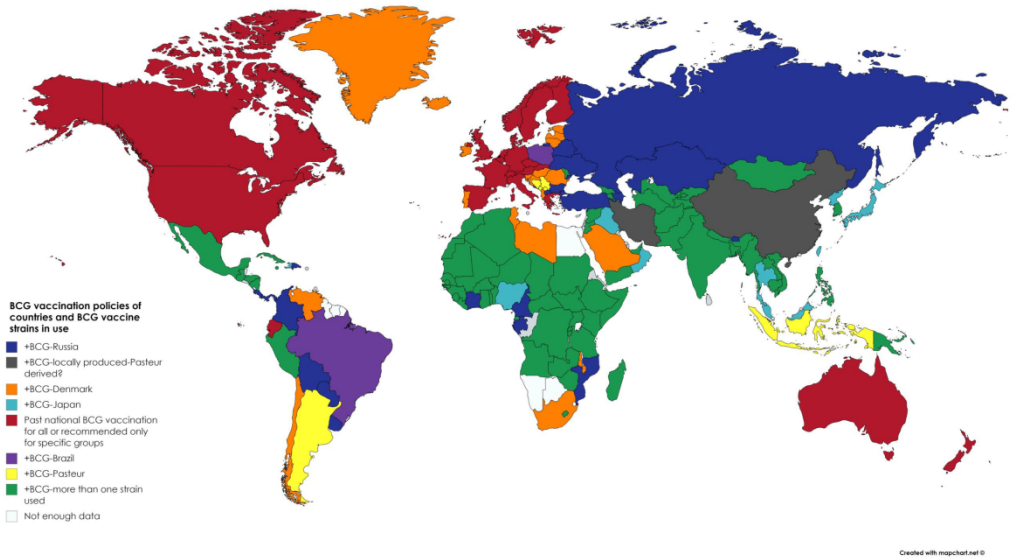

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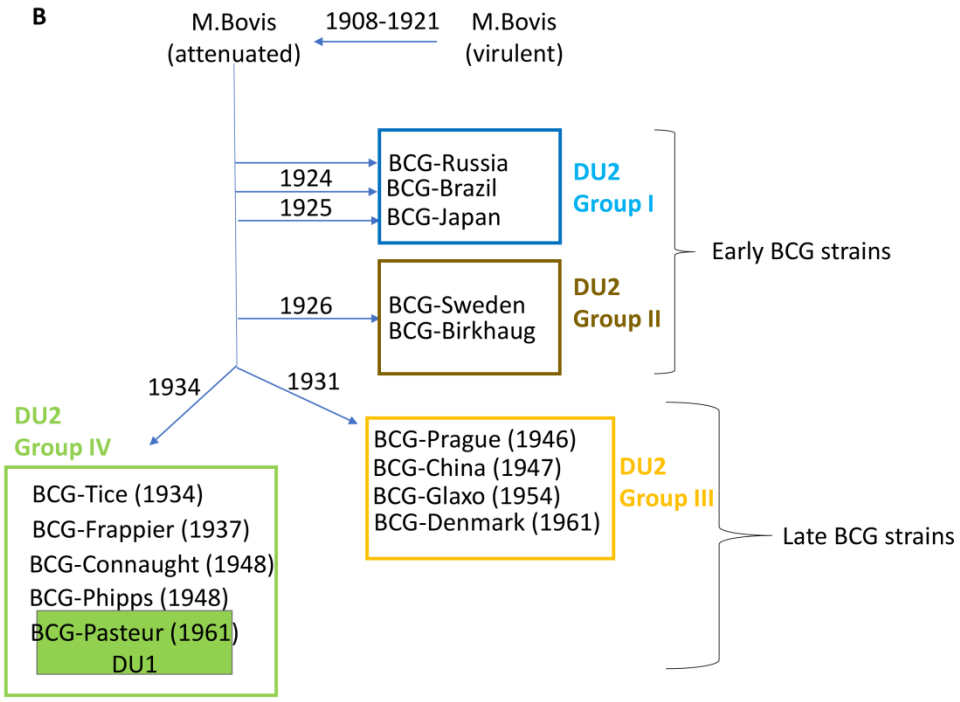

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