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Coronavirus Pandemic

Could "trained immunity" be induced by live attenuated vaccines protect against COVID-19? Review of available evidence

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Abstract

Coronavirus disease 2019 (COVID-19) represents a severe global public health threat. Caused by SARS-Cov-2, COVID-19 is characterized by high transmission rate that correlates with high viral load. The full clinical spectrum of the illness, the prevalence rates of mild symptomatic and asymptomatic cases, and the case fatality rates are still poorly understood, highlighting the importance of early preventive measures. Unfortunately, appropriate vaccination against SARS-Cov-2 is not yet available. Unless a target vaccine is developed, COVID-19 impacts will be devastating.

"Trained immunity" (TI), which could be induced by live attenuated vaccines (LAVs), is a potential public health preventive approach to boost the host immune system. Trained innate immune cells demonstrated phenotypical and functional changes leading them to acquire immunological memory and amplify their responses against subsequent infections. This phenomenon could have important public health preventive implications by harnessing the early immune responses against COVID-19, restricting its progression, and suppressing its infectivity.

Some LAVs have induced a broad, nonspecific, protection against unrelated pathogens and decreased mortality from conditions other than the targeted infectious diseases. This review summarizes the relevant literature and 1) emphasizes the role of available LAVs as potential stimulants for TI and 2) proposes this phenomenon as a potential preventive approach against COVID-19 that needs thoughtful consideration and further investigation. Clinical trials in this field are then urgently needed in line of vaccine and treatment unavailability. This is specifically true when considering two evolving scenarios; the virus spread may not diminish with warm weather, and that it will erupt a second-hit severe outbreak next winter.

Key words: live attenuated vaccines; COVID-19; trained immunity; review.

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Immune Responses to Coronaviruses

The immune response to invading pathogens passes through multiple phases. The innate immune system is an essential early host defense against coronaviruses. The innate immune cells, such as macrophages and dendritic cells (DCs), are induced through pattern recognition receptors (PRRs) when they recognize the invading viral pathogen–associated molecular patterns (PAMPs). Different known PRRs are involved including Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), C-type lectin-like receptors (CTLRs), and free-molecule receptors in cytoplasm. Induced PRRs activate downstream signaling pathways and release interferons (IFNs), the host's major antiviral molecules. IFNs are divided into three families, types I and III IFNs are key antiviral effectors in the innate response. Type II IFNs, produced by specialized immune cells, help in the inhibition of viral replication, stimulate and modulate the immune system [1–3].

Limited information is available on the host innate immune response in COVID-19. It is believed that SARS-CoV-2, similar to other (re-)emerging coronaviruses (SARS-CoV and MERS-CoV), developed multiple mechanisms to evade or antagonize IFNs response. Without this innate defense mechanism, which primarily restricts virus replication, the infection might progress. This IFNs dampening mechanism is correlated with the disease severity [4–7].

COVID-19 Clinical Presentation Spectrum

The symptomatic infection with SARS-CoV-2 has a spectrum of severity. Based on a report from the Chinese Center for Disease Control and Prevention on approximately 44,500 COVID-19 confirmed cases, 81% of patients had mild symptoms. Severe symptoms,

such as dyspnea and hypoxia, were reported in 14% of cases while 5% of cases showed signs of critical illness (respiratory failure or multi-organ dysfunction). The fatality rate, which all occurred among critical cases, was estimated at 2.3% [8]. A study of 138 patients hospitalized with SARS-CoV-2 pneumonia in Wuhan/China reported that the median days to develop severe illness after the onset of symptoms was five days, and hospitalization occurred after a median of seven days of symptoms [9].

Striking Viral Load

Upper respiratory specimens from COVID-19 patients showed very high viral RNA level soon after symptom onset, and peak concentrations were reached before day five. The viral load was higher in the early stage of illness compared with the later stage. This raises speculations that disease transmission might be more likely in the early mild stage of the infection [10]. Furthermore, respiratory specimens from patients with COVID-19 had the highest viral load near presentation compared to SARS patients. This could explain the fast-spreading nature of COVID-19. Significant positive correlation between age and peak viral load was also reported. While median initial and peak viral loads in severe cases were higher than those in mild cases, the difference was not statistically significant [11].

The emerging "Trained Immunity" concept

"Trained Immunity", defined as "a heightened response to a secondary infection that can be exerted both toward the same microorganism and a different one (cross-protection)", is an emerging concept [12]. Based on this concept, it is suggested that inducing training in innate immune cells promotes host resistance against a wide spectrum of pathogens. In order to investigate into this proposition, we ought to run a comprehensive literature review of the relevant literature. For that, our search has focused on the following key words using the PubMed database: Trained immunity, innate immunity, BCG vaccine, live attenuated vaccines, and COVID-19. Articles discussing "trained immunity" and the role of live attenuated vaccines in enhancing trained immunity were used to formulate the principle discussion points of this review.

Epidemiological evidence showed that some live attenuated vaccines (LAVs) induce heterologous or non-specific broad protective effects in addition to their diseases-specific effects. This makes such vaccines good candidates to promote "Trained immunity" effects and introduce their use as a potential Trained Immunity-Based Vaccines (TIbV) [13–15]. Trained innate immune cells are believed to preserve a primed functional state for a long time (up to months) [16]. This introduces an amplified immune response period which could provide a protection from bystander pathogens encountered by the host during this timeframe [13].

Until today, the mechanism of induced trained immunity is not fully investigated. It is speculated that quantitative enhancement of the innate immune responses and phenotyping changes in the innate immune cell subpopulations play an important role in this mechanism. Macrophages and NK cells are the most likely involved in the induced phenotyping changes, such as changes of PRR expression on cell membrane and alteration in their function of phagocytosis and cytokines production [12,17].

Studies on the immunological mechanisms responsible for post-BCG vaccination induced trained immunity demonstrated that BCG vaccination induced NOD2-dependent epigenetic reprogramming of monocytes through modulation of histone modifications. This resulted in increased expression of PRRs on the monocytes (CD11b, CD14, TLR4), and higher cytokine production in response to non-related pathogens. Interestingly, this enhanced function of circulating monocytes lasted between 3 months and one year after vaccination [18,19]. NK cells have also been influenced by BCG vaccination and showed enhanced proinflammatory cytokine production as a response to secondary pathogens [20]. Previously-activated NK cells were reported to acquire memory-like properties which result in a population of experienced NK cells with amplified function not dependent on constant stimulation [21].

Available vaccines with potential trained immunity effects

Research assessing the broad, non-specific, effect of LAVs are presented in Table 1. The most prominent example of LAVs that showed clinical and immunological non-specific effects beyond its targeted disease is *Bacillus Calmette–Guérin* (BCG). BCG is a live attenuated *Mycobacterium bovis* strain vaccine primarily used against tuberculosis.

In a randomized placebo-controlled human challenge study, BCG was found to provide protection against non-related viral infections after inducing epigenetic and functional reprogramming in human monocytes [22]. Multiple observational studies reported by the World Health Organization (WHO) Strategic Advisory Group of Experts on Immunization concluded that neonatal immunization with BCG

reduces all-cause mortality in the first month of life including mortality from sepsis and respiratory infections [23]. Interestingly, the same report stated that "The non-specific reduction in mortality following administration of the BCG vaccine became less pronounced as time progressed and as more children in the 'delayed BCG' group received their BCG vaccine". This provides further evidence of potent non-specific effects of the BCG vaccine [23]. The established link between BCG vaccination and reduced risks of acute lower respiratory infections (ALRI) among a large sample of children across multiple countries over a period of 25 years was further provided [24]. In consistence with the previous evidence, the incidence of respiratory syncytial virus associated ALRI were found to be lower among BCG vaccinated infants in Guinea-Bissau compared with unvaccinated infants [25]. This has been also supported by another retrospective analysis of epidemiological data from Spain, showing that BCG vaccination at birth may

Table 1. Summary of Research Assessing the Broad, Non-specific, Effects of Live Attenuated Vaccines.

Ref. No.	Author (year)	Vaccine	Result (vaccine effect)
18	Kleinnijenhuis J (2012)	BCG	✓ BCG induces trained immunity and non-specific protection from infections through epigenetic reprogramming of innate immune cells. ✓ Trained immunity induced through the NOD2 receptor and mediated by increased histone 3 lysine 4 trimethylation. ✓ Increased expression of PRRs on the monocytes (CD11b, CD14, TLR4). ✓ Sevenfold increase in the production of IFN-γ and twofold enhanced release of monocyte derived cytokines, such as TNF and IL-1β, in response to unrelated pathogens. ✓ The enhanced function of circulating monocytes persisted for at least 3 months after vaccination.
19	Kleinnijenhuis J (2014)	BCG	✓ BCG induces sustained changes in the immune system associated with a nonspecific response to infections. ✓ Monocytes recovered 1 year after BCG vaccination had an increased expression of pattern recognition receptors such as CD14, Toll-like receptor 4 (TLR4) and mannose receptor. ✓ Corresponding increase in pro-inflammatory cytokine production after stimulation with the TLR4 ligand lipopolysaccharide.
22	Arts RJW (2018)		✓ BCG induces epigenetic reprogramming in human monocytes *in vivo*, followed by functional reprogramming and protection against non-related viral infections, with a key role for IL-1β as a mediator of trained immunity responses.
23	Higgins (2014)	BCG	✓ BCG vaccine may reduce risk of all-cause mortality.
24	Hollm-Delgado MG (2014)	BCG	✓ Children vaccinated with BCG had a significantly lower risk of suspected acute lower respiratory infections (ALRI).
25	Stensballe LG (2005)	BCG	✓ BCG vaccination may have a non-targeted protective effect against ALRI and respiratory syncytial virus in infants in Guinea-Bissau.
27	Ritz, N (2013)	BCG	✓ BCG immunization at birth influences the antibody response to routine immunizations administered later in infancy.
28	Ota MO (2002)	BCG	✓ BCG influences the immune response to unrelated Ags in early life, likely through its influence on the maturation of dendritic cells. ✓ BCG priming markedly increased the cellular and Ab responses to the hepatitis B vaccine. ✓ The effect of BCG was apparent at the systemic level, as it increased the Ab response to oral polio vaccine.
30	Piedra PA (2007)	LAIV	✓ Trivalent LAIV provided protection against circulating influenza virus which is antigenically distinct from the vaccine strain. ✓ LAIV provided indirect effectiveness against medically attended acute respiratory illness in children 5 to 11 and adults 35 to 44 years of age. ✓ LAIV provides protection against influenza by both innate and adaptive immune mechanisms.
31	Chen GL (2011)	s-LAIV	✓ Prior immunization with s-LAIV primed mice for a robust response to p-H1N1 and led to complete protection in the upper respiratory tract.
32	Lee YJ (2018)	CAIVs	✓ CAIVs provided non-specific cross-protection against respiratory syncytial virus (RSV). ✓ RSV replication was significantly reduced when CAIVs was administered before RSV infection without any RSV-specific antibody responses.
33	Pennington SH (2019)	Ty21a	✓ Ty21a induced changes to monocyte phenotype/function which was observed for at least 3 months. ✓ It boosts the innate and adaptive immune cell cytokine production in response to stimulation with unrelated non-vaccine antigens. ✓ Enhanced cytokines response observed over the 6-month study period.
35	Kristensen I (2000)	MV	✓ MV was associated with better survival among infants in Guinea-Bissau
34	Aaby, P (2012)	MV	✓ Measles vaccination may significantly improve child survival and provide a solid basis of immunity.
36	Aaby P (2010)	MV	✓ MV reduced all-cause childhood mortality
37	Veirum JE (2005)	MV	✓ MV was associated with reduced mortality from diseases other than measles.
38	Mayr A (2004)	Vaccina Vaccine	✓ Protection against measles, scarlet fever, whooping cough, and syphilis.

BCG: Bacille Calmette-Guerin, NOD2: Nucleotide-binding oligomerization domain-containing protein 2, CD11b: Cluster of Differentiation 11b, CD14: Cluster of Differentiation 14, TLR4:Toll-like receptor 4, PRRs: P*attern recognition receptors*, IFN-γ: Interferon gamma, TNF: Tumor necrosis factor, IL-1β: Interleukin 1 beta, ALRI: Acute lower respiratory infections, Ags: Antigens, Ab: Antibodies, LAIV: Live attenuated influenza vaccine, s-LAIV: seasonal Live attenuated influenza vaccine, p-H1N1: pandemic Hemagglutinin 1 Neuraminidases 1, CAIVs: Cold-adapted, live attenuated influenza vaccine, RSV: Respiratory syncytial virus, Ty21a: Typhoid Ty21a strain vaccine, MV: Measles Vaccine.

decrease hospitalization due to respiratory infections and sepsis that are not related to tuberculosis through heterologous protection [26].

The influence of BCG immunization at birth on the subsequent antibody (Ab) response to routine immunizations with other vaccines was also investigated. The concentrations of antibodies against those vaccines were reported to be higher in the BCG-immunized group compared to the non-BCG-immunized group [27]. In this regard, administration of BCG at the time of hepatitis B vaccine (HBV) priming at birth and then at two months of age with booster HBV dose, markedly increased the cytokine response (IL-5, IL-13, with or without significant influence on IFN- γ), lymphocyte proliferation, as well as Ab responses to HBV compared with infants who had not received BCG. Similar marked influence of BCG was noted on Ab response to oral Polio virus vaccine in infants who received both vaccines at the same time at age of two months [28].

As part of the national immunization programs in most developing countries, BCG was suggested as an explanation of the lower number of cases compared to developed countries utilizing an ecological study design. The same study, however, warranted that clinical trials of BCG vaccine are still needed to establish the beneficial role of BCG in COVID-19 patients, as suggested by the ecological epidemiological data, especially in countries where universal BCG vaccination policy is not available [29].

Trivalent live attenuated influenza vaccine (LAIV-T) had significantly reduced medically attended acute respiratory illness incidence rates in children during the 2003 influenza outbreak, compared to the children who received trivalent inactivated influenza vaccine. This, further, provides an evidence that stimulated innate antiviral response produced by replication of LAIV-T in the host may confer protection from illnesses that are associated with influenza and other bystander respiratory viruses during the first weeks of vaccination [30].

In animal studies, seasonal live attenuated influenza vaccine (s-LAIV) induced very effective priming for stronger response to the monovalent pandemic H1N1 (p-H1N1) vaccine, which is genetically and antigenically distinct virus. The robust immune response led to complete protection in the upper respiratory tract and 25,000 fold reduction of the p-H1N1 virus in the lower respiratory tract on day two post challenge. This protection scenario was not observed after exposure to seasonal trivalent inactivated vaccine (s-TIV), before p-H1N1 vaccine administration [31]. Similarly, cold-adapted, live attenuated, influenza vaccine (CAIV) induced early innate immune responses in the respiratory tracts, restricted RSV replication, and amplified the early inflammatory cytokines following RSV challenge [32].

Vaccination with live attenuated *Salmonella Typhi* strain Ty21a**,** a low cost and extremely well tolerated vaccine, could provide broad non-specific beneficial effects. Ty21a showed a capacity to generate innate immune memory by inducing up-regulation of monocytes surface receptors and mount cytokine production of various cell populations to subsequently encountered pathogens. These changes could generate resistance to other types of infection [33].

Another vaccine provided to children and believed to introduce a broad spectrum, non-specific, prevention against diseases not originally intended in the vaccine itself, is the measles vaccine. Observational results on the mortality impact of Measles Vaccine (MV) suggested that the substantial mortality reduction in vaccinated children cannot be explained by the prevention of measles infection alone [34,35]. It is believed that the non-specific protection of MV led to prevention of non-measles-related mortality. In a randomized controlled trial to measure the effect of MV on all-cause childhood mortality, children vaccinated against measles had a significant reduction in hospital admissions, particularly admissions due to pneumonia [36]. Furthermore, the protective effect of MV was obvious for children with pneumonia as measles vaccinated children with pneumonia had a lower mortality than measles-unvaccinated children with pneumonia [37].

Vaccination against smallpox, introduced long time ago and made from a virus called vaccinia (live vaccina virus), was reported to confer protection against measles, scarlet fever, whooping cough, and syphilis when compared with non-vaccinated individuals [38].

Point of View

Evidence of the broad, nonspecific, protection provided by some LAVs against a range of pathogens are of primary clinical interest. The enhanced heterologous immunity, established by these vaccines, increases host resistance to re-infection or boost the ability of the immune system to handle subsequent infections. This concept of trained immunity has much needed benefits for COVID-19 and could carry beneficial consequences achieved by restricting viral replication in the early stage of the disease to slow down its progression. Viral load suppression reduces high transmission (infectivity) at an early stage, even before

appearance of the symptoms. While heightened activation state of immunity after administration of LAVs may be transitory or long-lasting, it may still provide protection against the infection until a COVID-specific vaccine is available or the disease season is over.

However, innate immunity responses were reported to diminish with advancing age, altered certain TLR-mediated responses and lower expression levels of some TLR in monocytes and DCs are observed in elderly. These observations, in addition to the suggesting defective regulation of the type I IFNs axis, lead to reduce antiviral response and increased susceptibility to infection in the elderly [39]. Under the trained immunity umbrella, promoting innate immune responses using the heterologous effects of vaccination could benefit the elderly population.

COVID-19 is a novel disease with a weak evidence-base on which to formulate clinical directions. So how do we move forward over the coming months, with all the uncertainty, change, and difficulties is questionable. Using available safe vaccines today, given the conceptual trained immunity concept, could dampen the morbidity and mortality of COVID-19. This is especially true for the hard hit population groups, who could benefit from it the most, such as the elderly, health care workers, and individuals who may be exposed to the disease, as well as individuals with certain comorbidities (immunocompromised patients, however, should not receive LAVs). LAVs as stimulants to trained immunity should be further investigated using clinical trials to establish a sound public health evidence to combat the spread of COVID-19. Initiating clinical trials within this field will provide insights into disease progression, guide public health preventive interventions and social policy guidance, and develop preventive measures for vulnerable populations. Simultaneously, it will build on available evidence presented in this review.

Finally, our review has focused mostly on the available literature linking LAVs and TI and not COVID-19 specific trained immunity. We however hinted to the possibility of a potential role of LAV in fighting COVID-19 while stressing the need for further investigation and considerations. In support of our propositions, recently published articles have focused on the potential beneficial effects of BCG vaccine in conferring a level of protection against COVID-19 and, at the same time, concluded that robust direct effects of these vaccines should be deferred until the final results of the ongoing clinical trials are available [40–42].

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