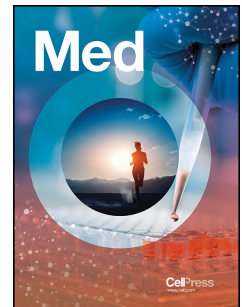


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Deadly COVID-19 Among the Elderly: Innate Immune Memory Helping those Most in Need

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Abstract:

Age is a key risk factor associated with the severity of symptoms caused by SARS-Co-V2 and there is an urgent need to reduce COVID-19 morbidity and mortality in elderly individuals. We discuss evidence suggesting that trained immunity elicited by BCG vaccination may improve immune responses and can serve as a strategy to combat COVID-19 in this population.

Keywords: trained immunity, immune response, elderly, BCG, COVID-19,

COVID-19 is a disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease spreads very quickly and became a pandemic short after it emerged China in December 2019. Depending on the patients' health conditions and age, COVID-19 clinical symptoms can range from an asymptomatic or mild disease to a very severe form. Pre-existing underlying health conditions such as diabetes, atherosclerosis, hypertension, cancer, immunocompromised states, chronic kidney disease and overweight/obesity substantially increase COVID-19 mortality and morbidity¹. In addition, COVID-19 severity drastically increases with advancing age^{2,3}. In patients with severe disease, COVID-19 induces a complex dysregulation of both innate and adaptive immune responses, that sometimes result in a combination of inappropriate systemic inflammation (leading to pulmonary edema and respiratory insufficiency), as well as defects in specific components of the immune responses (e.g. interferon pathway defects, lymphopenia), contributing to disease severity^{1,4}. Older people have a higher chance of developing a systemic inflammation when encountering COVID-19⁴.

Aging as a risk factor of COVID-19 progression

Aging is one of the main risk factors for increased susceptibility to many infectious diseases. Systematic analysis of more than 140 databases on 19 viral and 13 bacterial diseases demonstrated that severity increases with age and clinical symptoms markedly worsen in older ages^{5,6}. Although the COVID-19 pandemic affects people across all ages, children and young adults 10-19 years old show very mild or no symptoms. In contrast, the elderly are the most vulnerable group to the disease and are often hospitalized and require intensive care unit support^{3,6}. Subsequently, the mortality rate among this age group is significantly higher than among younger or middle-aged groups. Individuals under 20 years of age are almost half as susceptible to COVID-19 as those over 20. Clinical symptoms increased dramatically to 57-82% in adults aged over 65-70 years⁶.

Increased susceptibility to disease is a complex and multifactorial process. Physiologic alterations affect many organs such as the skin, epithelium and mucosal tissue. In addition, poor disease outcome is closely associated with the immune system weakening during aging² (**Figure 1**). Age-associated changes in both lymphocytes functions and proportions have been linked to chronic diseases. Lymphoid tissues deteriorate with rising age and fail to support lymphocyte hemostasis, which result in less T and B cells and finally less robust cellular and humoral immune responses. Evidences also shows that aging causes hematopoiesis shift toward myelopoiesis from lymphoid to myeloid progenitors. This may contribute to lower lymphoid cell numbers and a higher number of myeloid derived cells such as monocytes/macrophages^{2,5}. With aging, the frequency of naïve CD4+ or CD8+ T cells decreases but the

number of memory CD4+ or CD8+ T cells increases or remains the same^{2,5,7}. Moreover, the normal function of TCR and cytokine production by T cells is also impaired⁷. All these changes in adaptive immunity agents lead to a decline in their ability to build a proper immune response against new antigens, and thus poor response to vaccination. This is referred to as immunosenescence (reduced proliferation) and exhaustion (reduced inflammatory mediators such as cytokines production)^{3,7}. Exhausted T cells continuously produce small amounts of proinflammatory cytokines such as IFN γ and TNF α , which induces low grade inflammation in bodies of older age³.

Age related defects in the adaptive immune system against COVID-19

Recently, Moderbacher *et. al.* showed that scarcity of all three arms of adaptive immunity, such as B cells (antibody producing cells), CD4+T cells (helper T cells), and CD8+T cells (cytotoxic, or killer, T cells) plays a crucial role in the severity of COVID-19 in the elderly². SARS-CoV-2-specific CD4+ and CD8+ T cell levels were significantly lower in acute COVID-19 samples in comparison to convalescent COVID-19 cases². The serological assays found that many types of circulating antibodies against RBD (Receptor Binding Domain), S (Spike protein), and N (Nucleocapsid protein) as well as neutralizing antibodies against full-length SARS-Cov-2 S protein were pronounced in both acute and convalescent COVID-19². These neutralizing antibodies were associated with a protective response against COVID-19 and were tested as vaccine candidates in non-human primates². Conversely, strong SARS-CoV-2- specific CD4+ or CD8+ T cell responses were associated with protection against the disease and milder symptoms in the acute phase. With aging, frequencies of naïve CD8+ and CD4+ T cells are dramatically reduced and their responses against diseases including COVID-19 significantly altered². In order to mount a well-defined immune response against a pathogen, the immune system uses highly coordinated processes involving many cells and mediators. Moderbacher and her colleagues demonstrated that the alteration in coordination of the immune system in older people is a reason why they cannot compete against the novel coronavirus². Impairment or loss of coordination of the CD4+ and CD8+ T cell responses and lack of proper coordination between the CD4+ T cell and antibody responses lead to a severe form of COVID-19 in the elderly. Interestingly, there was a strong association between low frequencies of naïve CD8+ and CD4+ T cells, severity of COVID-19, and age. On the other hand, acute cases which had high IFN γ - producing CD8+ T cells exhibited mild symptoms². Intriguingly, pairwise analysis revealed a positive association between disease severity, antigen specific antibodies and CD4+ and CD8+ T cells responses, but neutralizing antibodies alone were not a factor to reduce disease symptoms. This suggests that an

efficient coordination between all three arms of the adaptive immunity is needed for protection against COVID-19, which is almost lacking in the elderly ².

Age associated defects in the innate immune system

Innate immunity is the first line of defense that responds immediately upon encountering a threat. It also plays a crucial role in initiating an effective adaptive immunity. The innate immune system is affected by age to a lesser degree. Adaptive immune cells respond to inflammatory stimuli in an antigen specific manner, whereas innate immune cells provide nonspecific defense against invading agents. Neutrophils, monocytes and natural killer (NKs) numbers are preserved, but the number of dendritic cells (DCs) is reduced with increasing age. However, function of monocytes, NKs and neutrophils such as chemotaxis and signal transduction are impaired in older individuals ⁸. Adaptive immune response induction requires the presentation of antigens to T cells by antigen-presenting cells (APCs) mainly DCs and higher number of DCs not only enhances the innate immune response but also can mediate activation of adaptive immune response against pathogens. On the other hand, impaired DCs function causes delayed T cell responses and ultimately lower anti-viral responses.

Age-associated decrease of Toll-like receptors (TLRs) such as TLR1, TLR3 expression could be another reason for weakening immune responses ⁷. Moreover, the expression of TLR7 in the lung is depleted with age, which may contribute to a poor immune response to COVID-19 in elderly patients ³. In addition, immune response related gene expression analysis revealed an age-related upregulation of innate immunity and downregulation of adaptive immunity which was associated with viral replication and severity of COVID-19 ³.

Dysregulation of innate immune responses plays a central role in the increase of peripheral cytokines and chemokines such as IL-1 β , IL-6, IL-8 and CXCL10, which are associated with immunopathogenesis and serious clinical signs in the late stages of severe SARS-CoV-2 infection ^{2,4}. Elderly individuals might experience a sustained activation of innate immunity and increased circulating inflammatory mediators, which together with low level of cytokines produced by T cells induces a chronic low-grade inflammation. It was noted that concentrations of inflammatory cytokines were increased with aging in the acute respiratory syndrome ⁴. Interestingly, Moderbacher *et. al.* also showed that CXCL10, IL-8 and IL-6 production was increased in the acute phase and positively associated with the severity of the disease. CXCL-10 also showed a strong negative correlation to SARS-CoV-2 specific CD4+ and CD8+ T cells suggesting that CXCL10 may serve as a surrogate marker in predicting poor CD4+ and CD8+ T cell response against COVID-19 ².

In elderly patients, failure to fight against SARS-CoV-2 infection is a complex process and in addition to a less efficient immune system, several other factors can play a role:

- Angiotensin-converting enzyme 2 (ACE2) is a highly specific receptor which facilitates SARS-CoV-2 entry into cells such as epithelial or alveolar cells⁵. The expression of ACE2 in the lung, nasal epithelium and other organs increases with age. Heightened receptor availability enhances the viral entry into the host cells and increases the number of infected cells. Therefore, the level of ACE2 expression can aggravate COVID-19 severity^{3,9}. Sajuthi *et. al.* found that IL-4, IL-5, and IL-13 production significantly reduces ACE2, which correlated with a less severe COVID-19, whereas type 1 IFN induction increased ACE2 expression⁹.

- Many elderly individuals suffer from underlying medical conditions and are therefore at a greater risk for a more severe disease progression.

- Shifting lymphoid cell fractions to predominantly memory cells increases the chances that these cells may cross react with new antigens, which can induce an unwanted immune reaction and lead to a severe form of the disease^{2,3}.

Trained immunity against COVID-19 in the elderly

In early life, children receive many vaccines against infectious diseases. Randomized trials have shown that vaccines such as the measles vaccine or *Bacillus Calmette-Guérin* (BCG) vaccine nonspecifically reduced overall child mortality due to acute respiratory tract infections¹. Nonspecific cross protection of vaccines against unrelated infectious diseases is mediated by epigenetic and metabolic changes in innate immune cells and is termed trained immunity or innate immune memory¹. Trained immunity can confer broader aspect of therapeutic strategies against infectious and noninfectious diseases such as atherosclerosis, the bladder cancer and destruction of cancer cells^{1,10}. Several investigations have reported a significant correlation between BCG vaccination and decreasing COVID-19 morbidity and mortality in countries where BCG vaccination is a part of the immunisation program (reviewed in¹)^{10,11}. These ecological studies are prone to bias due to several confounding factors such as genetic background, geographical regions, diagnostic tests, type of data collection and control regulations^{1,10}. Moreover, Nonspecific protection induced by BCG vaccination varies depending on BCG strains, dose, administration route and manufacturing which can explain lack or less efficient protection in some populations^{1,10}. Escobar *et. al.* found that after mitigating multiple confounding factors, every 10% increase in the BCG index was associated with a 10.4% reduction in COVID-19 mortality¹⁰. These data

warrant the further investigation of the concept of nonspecific effects of live attenuated vaccines in prospective randomized trials.

Vaccination is the most effective way to prevent infectious diseases. However, in general vaccines are less effective in older people. For example, the influenza vaccine efficiency is reduced to about 20-60% in older population ⁶⁻⁸. It is therefore an emerging task to protect the most vulnerable against the diseases. Development of an effective vaccine against COVID-19 has resulted in exciting news regarding several potential candidates with a good effectiveness and safety profile, at least in young individuals, but it is an additional concern to increase efficacy of the potential vaccine in the elderly. Immune system and vaccine effectiveness in the elderly can be boosted by several ways. Changing the administration route or the use of adjuvants can increase the efficiency of vaccines ⁷. Another strategy is using a trained immunity approach to boost the immune system and protect the older population against COVID-19. Administration of microbial compounds such as BCG or β -glucan induced a long-term memory and enhanced immune responses in monocytes/macrophages, DCs and NK cells in older people ⁸ (**Figure 2**). Randomized controlled trial showed that BCG vaccination was able to reprogram the bone marrow HSCs and upregulate myeloid and granulocytic lineage associated transcripts 90 days after vaccination ¹². Interestingly, BCG vaccination induced persistent epigenetic changes in circulating CD14+ monocytes 90 days post administration. This suggests that BCG induced transcriptomic changes in hematopoietic stem/progenitor cells (HSPCs) passed to the major effector cells of peripheral blood mononuclear cells (PBMCs) such monocytes ¹².

A recent paper by Giamarellos-Bourboulis *et al.* demonstrated the impact of BCG vaccination on the modulation of immune responses and prevention of respiratory infections in the older patients ¹¹. In this randomized trial, elderly hospitalized patients were vaccinated on the day of their hospital discharge with a single dose of a placebo or BCG. Vaccination drastically decreased respiratory infection incidence by 80% in the BCG vaccinated group in comparison to the placebo group during the 12 months of follow up, suggesting a long-term nonspecific memory effect of BCG ¹¹. Thus, while the defective adaptive immune memory might lead to lower responses to vaccination, gain of innate immune memory could be a key to the survival of the elderly. As expected, the PBMCs of the immunized group showed a significantly enhanced IL-6 and TNF α cytokine production after Pam3Cys restimulation. These results were coupled with the increased levels of H3K27ac at the regions of IL-6 and TNF α promoters, suggesting sustained histone modification upon BCG administration ¹¹. The mechanism behind this effect can be explained by transcriptional and functional changes at the level of myeloid bone marrow progenitors. Cirovic *et al.* showed that BCG vaccination upregulates expression of genes associated with

neutrophils and mononuclear phagocytic cells within HSPCs 90 days post vaccination¹². Number of neutrophils increases dramatically in COVID-19 but their functions in older patients are impaired. Thus, BCG vaccination might improve neutrophils functions and their antiviral activity. BCG vaccination also induced IFN γ production after *M. tuberculosis* restimulation, which indicates the activation of NK cells or adaptive immune responses¹¹. NK cells are crucial for an efficient antiviral immunity. These cells identify and eliminate infected cells and produce cytokines or chemokines such as interferon. Interestingly, recent studies have implicated that BCG vaccination improves NK cells functions¹³. This suggests that BCG treatment can revive their antiviral ability, which was altered due to aging.

Cirovic *et. al.* showed that BCG induced IL-1 β production and enrichment of pathways related to IFN α and IFN γ response signaling in PBMCs, 90 days after administration¹². In many viral infections, T cells play an important role in the control and clearance of an acute infection. Coordination and synergistic effect of the innate and the adaptive immune response is the most efficient way to fight against SARS-CoV-2. Interestingly, there were no remarkable changes in circulating monocyte, granulocyte, or lymphocyte counts. Moreover, there were no significant increase in the level of inflammatory mediators such as IL-6 and IL-18. This indicates that in fact there was no systemic inflammation after BCG vaccination¹¹. Previously, it was shown that BCG decreases systemic chronic inflammation by improving cell responsiveness¹⁴. Lowering systemic inflammation and enhancing innate and adaptive immunity during COVID-19 might be an important step to lower mortality rate due to cytokine storm syndrome.

Conclusion:

Considering the impairment of the elderly immune system, these data suggest that BCG is able to recover both innate immune system and the T cell immune responses and significantly influences the level of immune responsiveness. BCG is able to induce adaptive and innate immune responses and have a beneficial effect on coordination of both systems, thus can result in more efficient and effective protection against COVID-19 in the older ages. Moreover, the prevalence of cancer diseases such as non-muscle-invasive bladder cancer and melanoma are significantly higher in older people and BCG administration against COVID-19 might have protective effects against those diseases, which is beyond its intended scope. It is important to study whether BCG administration can also restore hematopoietic progenitor cell functions and frequency in aged humans. It has been shown that trained immune memory can also be induced in non-immune cells, such as stromal and epithelial cells¹. Epithelial cells

are the first host cells to be infected by SARS-CoV-2 that is why they are particularly important target cells for the induction of innate immune memory.

In conclusion, BCG-induced innate immune memory can improve antiviral-host defense in older people. Several epidemiological and proof-of-principle studies have shown that BCG vaccination prior to other vaccines boosts the efficiency¹⁵. This suggests that building a pre-existing immunological memory not only could be a strategy to protect the elderly against new infection, but also be a tool to strengthen their immune system capabilities and increase the efficacy of vaccines. It is very important to consider that currently, BCG is used as a routine neonatal vaccination policy in 152 countries and also is used as an immunotherapy for non-muscle-invasive bladder cancer and melanoma worldwide^{1,10}. If BCG vaccination is considered as an approach to prevent COVID-19, the new application should not interfere with the routine infant vaccination program or immunotherapy of adult patients. A well-defined measure and careful evaluation is required to manage any possible shortage in the vaccine supply. Altogether, BCG vaccine is safe and can protect the elderly against infection without inducing serious adverse effects and systemic inflammation. Considering the effect of BCG on the reduction of COVID-19 severity and death, nonspecific boosting of immune responses by BCG or other microbial compounds could be considered as a transient but a powerful approach to protect the vulnerable population. Likewise, this strategy can decrease mortality and morbidity rates of COVID-19 and other infectious diseases as a 'bridge vaccination' between the occurrence of a new pathogen and the development and deployment of novel specific vaccines.

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Declaration of Interests

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

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Figure 1: COVID-19 alters innate and adaptive immune systems in the adult and the elderly. Age-related alteration in immune responses increases susceptibility to SARS-CoV-2 infection. Changes in innate and adaptive immune systems are indicated in adults and older COVID-19 patients. Intensity of the color indicates direction of changes where lighter color displays decrease in phenotypic value.

Figure 2: BCG vaccination modulates innate and adaptive immune system in the elderly. Age-related alterations in the immune system contribute to the increased susceptibility to diseases in the elderly. BCG vaccination improves immune systems in terms of cell numbers and functions in both innate and adaptive immune systems. Changes in immune cells are indicated before and after BCG vaccination in the elderly. Intensity of the color indicates direction of changes where lighter color displays decrease in phenotypic value.

Adults	Cells	Elderly
Increased numbers, higher NET-associated genes	Neutrophils	Higher neutrophil lymphocyte ratio
Increased number and cytokine production	Monocytes	Higher number, upregulation of cytokine responses
Decreased number	DCs	Dysfunctional dendritic cells
Decreased number in severe form	NK cells	Decreased numbers and altered function
Less number, less proliferation, more TNF α than IFN γ production	naïve CD4+ T cells	Decreased number in severe form, Less number in acute patients than convalescent
Decreased number, less proliferation Impaired responses, more granzyme B and perforin production than IFN γ	naïve CD8+ T cells	Less number and impaired function, higher senescent and exhaustion marker
Decreased number, higher IgM, IgG and IgA production	B cells	Less naïve B cell, Less antibody production in severe form
Higher number of effector memory cells in convalescent, lower number of central memory cells in severe form	Memory CD4 cells	Increased number of effector memory cells, higher cross reactivity
Decreased number of central memory cells in severe form	Memory CD8+ T cells	Less central and effector memory cells, higher memory/naïve cell ratio
Decreased number in severe form	Memory B cells	Higher memory/naïve B cell ratio

Elderly	Cells	Elderly + BCG
Less TLRs expression, Low metabolism, less phagocytosis and migration, higher NETs formation	Neutrophils	Upregulation of transcriptomic associated with phagocytosis, long term phenotypic changes
Less TLRs expression, Low metabolism, less phagocytosis, migration and cytokine production	Monocytes/ Macrophages	Upregulation of inflammatory response, term epigenetics and metabolic changes
Less DCs and decreased cytokines secretion, weak TLR4 signaling	DCs	Increased survival, numbers and MHCII expression
Low Proliferation, low cytokine production, lower cytotoxic activity	NK cells	pro-inflammatory cytokine production, epigenetic and metabolic changes
Low number, less proliferation, less TCR diversity and function, low cytokine production	naïve CD4+ T cells	Increased number and function
Low number, low antigen presentation, less antibody production	naïve CD8+ T cells	Higher number
Low number, low antigen presentation, less antibody production	B cells	Increase level of IgG and IgA
Higher number, higher cross reactivity	Memory CD4 cells	Increased number of effector memory cells
Higher number, higher chance of cross reactivity	Memory CD8+ T cells	Higher number central and effector memory cells
Less lymphoid progenitors	HSCs	Myelopoiesis, persistent epigenetic changes