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Enhancing innate immunity against virus in times of COVID-19: Trying to untangle facts from fictions

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ABSTRACT

Introduction: In light of the current COVID-19 pandemic, during which the world is confronted with a new, highly contagious virus that suppresses innate immunity as one of its initial virulence mechanisms, thus escaping from first-line human defense mechanisms, enhancing innate immunity seems a good preventive strategy.

Methods: Without the intention to write an official systematic review, but more to give an overview of possible strategies, in this review article we discuss several interventions that might stimulate innate immunity and thus our defense against (viral) respiratory tract infections. Some of these interventions can also stimulate the adaptive T- and B-cell responses, but our main focus is on the innate part of immunity. We divide the reviewed interventions into: 1) lifestyle related (exercise, >7 h sleep, forest walking, meditation/mindfulness, vitamin supplementation); 2) Non-specific immune stimulants (letting fever advance, bacterial vaccines, probiotics, dialyzable leukocyte extract, pidotimod), and 3) specific vaccines with heterologous effect (BCG vaccine, mumps-measles-rubeola vaccine, etc).

Results: For each of these interventions we briefly comment on their definition, possible mechanisms and evidence of clinical efficacy or lack of it, especially focusing on respiratory tract infections, viral infections, and eventually a reduced mortality in severe respiratory infections in the intensive care unit. At the end, a summary table demonstrates the best trials supporting (or not) clinical evidence.

Conclusion: Several interventions have some degree of evidence for enhancing the innate immune response and thus conveying possible benefit, but specific trials in COVID-19 should be conducted to support solid recommendations.

Keywords: COVID-19, Immune response, Innate, Exercise, Mindfulness, Sleep, Bacterial vaccine, Trained immunity, Bacillus calmette-guérin, MMR, NK-Cell

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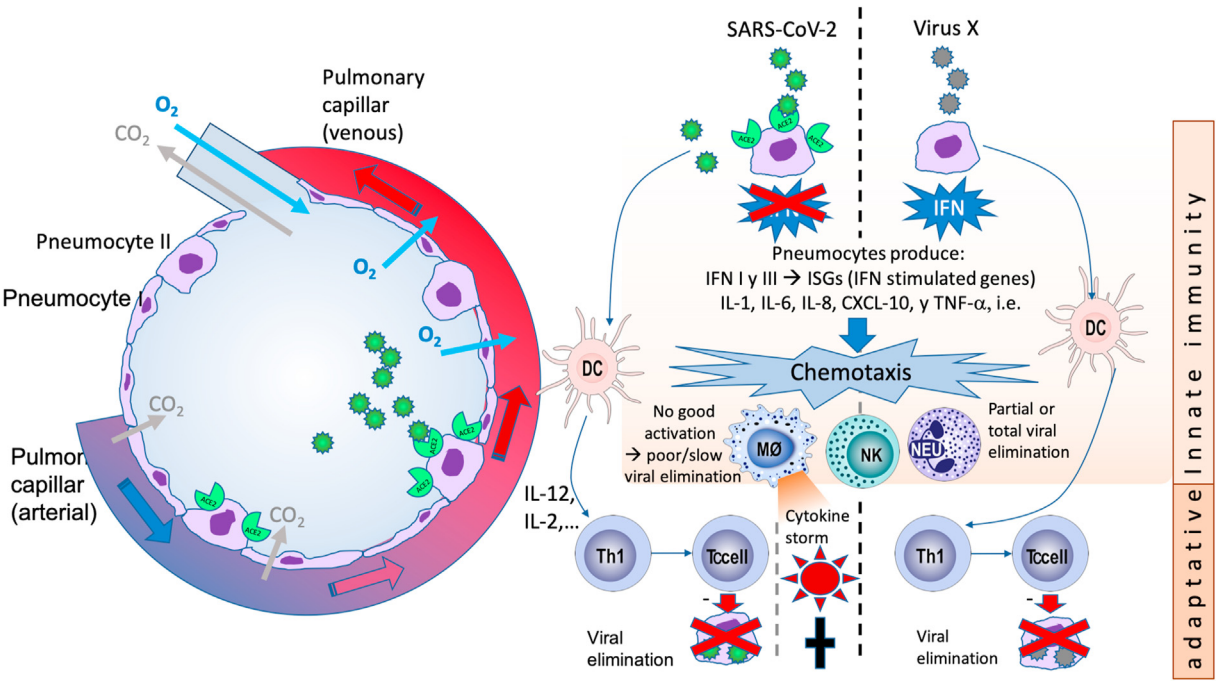

Fig. 1 SARS-CoV-2 infects type 2 pneumocytes by entering the cells via the ACE2 receptor on their surfaces. Unlike the normal anti-viral response with increased IFN type I and III and with it the activation of genes stimulated by IFN in adjacent cells and thereby increasing its anti-viral defense, the coronavirus has mechanisms that lower this anti-viral innate defense mechanism by interfering with IFN production and its effects. In addition, chemotactic molecules are released in a viral infection that attract macrophages (MØ), natural killer (NK) cells, and neutrophils. This reaction is not fully achieved during early infection with coronavirus, so the initial innate immune response appears incomplete and slow. After this first innate response, adaptive immunity is triggered via activation of dendritic cells (DC) that stimulate specific Th1 lymphocytes, which in turn activate cytotoxic T cells (Tccell) to eliminate infected cells, in the more advanced stages of the infection, along with plasma cell development and the production of antiviral IgM and IgG antibodies (not shown). However, in some patients with COVID-19 at this stage a dysregulated activation of macrophages (ie, by IL6) is seen, causing the feared cytokine storm.

INTRODUCTION

Taking a closer look at the evolution of the coronavirus disease 2019 (COVID-19) pandemic and learning from previous historical events with similar conditions, such as the Spanish flu (an H1N1 influenza virus) pandemic in 1918–1919, it is very probable that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) shall still be around for a while.[1] Due to its high basic reproduction number (R_0), close to 3.5 under natural conditions without social distancing,[2,3] the virus shall probably keep on infecting human beings until an effective vaccine has been developed, herd immunity (possibly both, cellular and humoral) has been built, or the virus has undergone loss-of-potency mutations.

At first contact with the respiratory mucosa, coronaviruses including SARS-Cov-2 enter the cells via the angiotensin converting enzime-2 (ACE2) receptor and, after transcription of its ribonucleic acid (RNA), produces structural and non-structural proteins. Among those proteins, the N-protein is capable of blocking the interferon (IFN) production and its effects on adjacent cells, see Fig. 1.[4] Thus, the first days after infection the virus subverts the host's initial innate immune response; this enables it to replicate without being noticed by the body. Once symptoms start, they are often aggressive with high fever and generalized malaise, as already many cells have been infected. Approximately 7 days after the onset of the initial symptoms is the decisive moment: the immune system eliminates the virus, giving rise to the patient's recovery, or the virus advances into the lungs, attacking specifically type 2 pneumocytes, and into the systemic circulation, causing destruction of the endothelium and oxidative stress. Further into the second week, in about 3-5% of all patients, this leads to an overactivation of the inflammatory response, specifically caused by interleukin (IL)-6 that activates macrophages, monocytes, and T-cells,

Box 1. Review content: Interventions that could enhance the innate immune response or improve the redox balance.

1. Life style factors
   a. Exercise
   b. Forest bathing
   c. Sleep
   d. Meditation/mindfulness
   e. Supplementation
      i. Vitamin D
      ii. Vitamin C
      iii. Zinc
      iv. Others
2. Non-specific immune stimulants
   a. Fever and hyperthermia
   b. Bacterial vaccines
   c. Probiotics
   d. Dialyzable leukocyte extract (DLE)
   e. Synthetic molecules (Pidotimod)
3. Specific vaccines with heterologous effect
   a. Bacillus Calmette-Guérin (BCG) vaccine
   b. Other heterologous vaccines

causing what some investigators describe as a "cytokine storm".[5] In this context it should be borne in mind that the cytokine levels in COVID-19 are 100 to 1000-fold lower than in the true cytokine storm syndrome described years back; and at this point it is not fully understood at what level there is an actual immune overactivation in COVID-19 and if so, what the prime site is.[6]

Summarizing, together with the total viral load and the adaptive immune response the role of the innate immune system in the outcome of COVID-19 seems of the utmost importance. When innate immune cells, natural killer (NK)-cells, macrophages, and neutrophils, would recognize virus infected mucosal cells right from the start, they could reduce viral replication and eliminate virus infected cells and SARS-Cov-2 would not be able to advance in the body – even less so, if the adaptive immune response would develop satisfactorily and take care of the remaining virus. And, at a later phase, when immune regulation would be able to balance immune activation better the immune over-activation could be avoided. Finally, a higher level of antioxidants in the patient's body could counterbalance the mechanisms by which the coronavirus causes endothelial damage. In each of these steps there might be an opportunity for intervention.

With this in mind, the question arises as to how the innate immune system could be reinforced, to enhance its preparedness to fight SARS-CoV-2 from the start and as a consequence to stimulate the cellular adaptive immune response further downstream. Although it has become clear that T-cells are key-players in the adaptive stage, with epitope pools of SARS-CoV-2 detecting $CD4^+$ and $CD8^+$ T cells in 100% and 70% of convalescent COVID patients[7] and also a special subpopulation of B lymphocytes, the B-1a cells that produce natural IgM in an autocrine manner, might have a special therapeutic potential,[8] we would like to focus our review on interventions that primarily stimulate and regulate the innate immune system.

Alongside, one could wonder if there are ways to enhance the redox balance and reduce oxidative stress. We present in several short chapters, see Box 1, different interventions that might help in these directions, discuss the evidence, and conclude on their practical usefulness.

LIFESTYLE FACTORS

First, we review some of the life style factors that might improve the innate immunity or the redox balance.

Exercise

Even though there are still no published clinical or experimental data about the role of exercise in the context of the COVID-19 pandemic, extrapolated evidence from previous studies shows that regular, moderate-to-vigorous exercise improves immune competency across the lifespan and may help to reduce the frequency of upper-respiratory tract infections (URTIs).[9,10] Importantly, this has

been observed also in older individuals (>65 year-olds) who regularly exercise, whose URTIs incidence per year has been inversely associated with the energy expenditure utilized during physical activity.[11,12]

There is growing scientific evidence showing that regular exercise may induce modifications of cell populations with antiviral properties in the bloodstream. Zamani et al observed that moderate exercise had an effect on cytokine responses: post-exercise peripheral blood mononuclear cell (PBMC) cultures showing a significant increase of IFN-γ and IL-12 compared to their paired pre-exercise samples, with a significant reduction of the level of IFN-γ after 2 months of post-exercise inactivity. Interestingly, IL-6 levels were not significantly affected by exercise.[13]

During a 40–60 min period of intense exercise, transient lymphocytosis occurs, typified by a sudden influx of both NK and $CD8^+$ T-cells (rising up to 10-fold and 2.5-fold, respectively), which favors an effector-memory immune phenotype.[14] This effect is governed by stimulation of beta-2-adrenergic receptors on the surface of lymphocytes (arising from adrenaline released during exercise), causing lymphocyte endothelial detachment and recirculation,[15] which also induces the expression of $CD4^+$ B-cells and regulatory T-cells.[16] Also, exercise helps to maintain immune homeostasis through bone marrow homing and increasing apoptosis of exhausted/senescent T-cells, thus stimulating the production and release of new progenitor cells (ie, IFN-producing $CD8^+$ T-cells).[14] Lastly, exercise has been associated with enhanced vaccination responses.[17]

Another relevant anti-inflammatory effect of constant exercise is the potential capacity to modulate the activation of TLRs signaling, as TLRs have been shown to be important in the innate immune response during a SARS-CoV infection.[18] It has been demonstrated that physical inactivity correlates with augmented TLRs activation and, conversely, that chronic exercise has an effect of decreasing cell-surface expression of TLRs on immune cells.[19] In addition, there is evidence showing that moderate, enduring exercise has the potential to reduce the activation of the NLRP3 inflammasome as well as IL-1β, a cytokine processed by the inflammasome.[20]

Conversely, some studies report that during prolonged periods of strenuous exercise (such as marathons) and after approximately 2 weeks following intense competitions, there is an immunodepression span (the "open window" period), related to a higher rate of URTI-like symptoms (mainly self-reported) vs those who undergo lower physical activity.[21,22] This theory, attributed to the consequences of psychological stress and excessive training,[23,24] is however highly debated today in light of a better understanding of the variability in the intensity-related response to physical activity, which depends largely on the functional capacity of the immune system. As a result, up-to-date perceptions on this topic tend to discard the concept of exercise-induced immunosuppression. In fact, it is widely accepted that during and after exercise, when performed regularly, the immune system is in a heightened state of immune surveillance and regulation.[25]

During the COVID-19 quarantine it is important to stay active and to exercise regularly (gradually increasing intensity), not only as a way to keep a healthy lifestyle, but also as an immunoprotective and immunoregulatory activity. It is possible that older adults (who are at higher risk of a severe SARS-CoV-2 infection) may obtain the greatest exercise-induced benefits to their immunological health.

Forest bathing (*Shinrin Yoku*)

In a series of several, subsequent small studies, Japanese investigators in cooperation with experts from the Stanford University have shown that walking in a forest seems to enhance NK cell activity, partly stimulated by volatile substances produced by trees (particularly phytoncides: antimicrobial essential oils from trees). The first experiment showed *in vitro* that phytoncides activate NK cell activity and augment NK cell granule content.[26] Then they confirmed this in peripheral blood lymphocytes of subjects submitted to a prospective trial of a 3-day, 2-night trip to the forest including three 2-h walks.[27] In a third case-control trial they demonstrated that the effect was not related to the walking tour, as they compared the rise in NK-cell activity after a 3-day tour to a small city with that after a 3-day tour to

the forest. Both tours included three 2-h walks, but NK-cell activity was only enhanced after the forest trip. Moreover, NK-cell activity stayed elevated one week and still somewhat 30 days after the tour.[28] In the last experiment investigators sought to confirm it is the inhalation of phytoncides, that cause the NK cell activation, by having study-subjects sleep in rooms where phytoncide was sprinkled in the air.[29] Even though all studies are small, confounders have been neatly corrected for.

Sleep

To date, no studies have been published that demonstrate a preventive effect of sleep against COVID-19. However, it has been proposed that a healthy lifestyle, with good sleep quality, could increase and modulate the functions of the immune system.[30]

A first mechanism is the optimization of the cytotoxic T cell response by functional down-regulation of the G protein coupled receptor alfa-subunits ($G\alpha_s$) receptor during sleep. This receptor has an immunosuppressive effect by inhibiting intercellular adhesion molecule type 1 (ICAM-1) and with it the function of the β2-integrins, essential for the ability of T-cells to adhere to their target cells (including those infected by virus) through the T-cell receptor (TCR). A paired *in vitro* study with cell cultures confirmed that, compared to wakefulness, sleep (8 h) significantly increased fluorescence uptake of ICAM-1 multimers in T cells, infected with cytomegalovirus or Epstein-Barr virus [$F(1, 9) = 4.1$; $P = 0.05$ and $F(1.8) = 6.0$; $P = 0.05$, respectively]. Interestingly, a 2-h sleep deprivation appeared to be sufficient to affect the adhesion capacity of T cells.[31]

Other studies have shown that sleep deprivation, both complete or only selective to the rapid eye movement (REM) stage, modifies various components of the innate immune system, such as dendritic cells (DCs) and the percentage of some subpopulations of T cells ($CD4^+$, $CD8^+$ and NK) and cytokine levels (IFN-γ, TNF-α and IL-1).[32–34] Specifically, in a clinical study in women 77 h of sleep deprivation (total and REM phase) resulted in a proinflammatory state and affected the cellular immune response, presenting changes in the production of IFNs and in the phagocytic activity. Sleep deprivation also decreased

	Cohen et al.	Prather et al.
Design	Prospective	Prospective
Number of volunteers	153	164
Age	21–55 years old	18–55 years old
Duration of the study	5 days	7 days
Monitoring	Duration and effectiveness of sleep	Duration and continuity of sleep
Objective sleep measurement	Actiography[a]	Actiography[a]
Results	OR = 2.94 (95% CI 1.18–7.30) of having flu symptoms in those who had <7 h of sleep	OR = 4.50 (95% CI 1.08–18.69) of having flu symptoms in those who slept < 5 h OR = 4.24 (95% CI, 1.08–16.71) those who slept 5–6 h vs. >7 h of sleep per night
Virus	Rhinovirus	Rhinovirus
Covariate Adjustment Results[b]	No effect	No effect

Table 1. Effect of sleep duration and common cold symptoms after experimental inoculation with rhinovirus. *OR: Odds ratio. a. Actigraphy: validated method for measuring objective l sleep parameters. b. Covariates: age, antibody levels before surgery, sex, body mass index, race, season of the year during the study, socio-economic level, educational level, income, smoking, physical activity, alcohol consumption, stress, kindness and lifestyle*

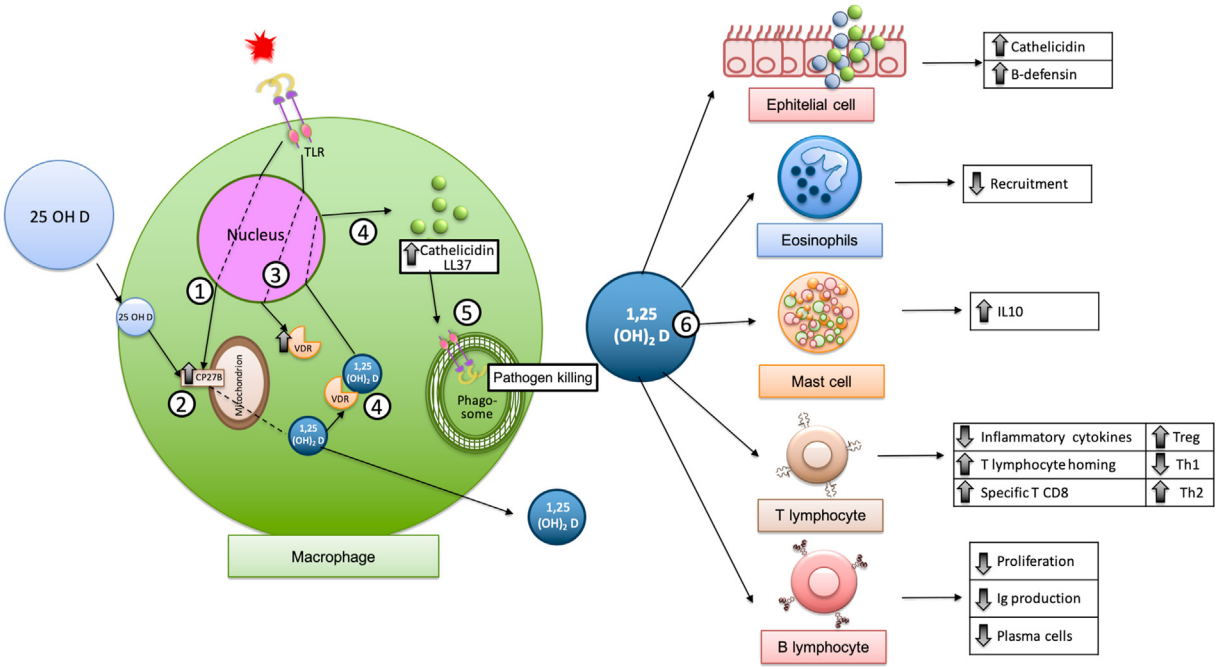

Fig. 2 The conversion of 25 OHD_2 to 1,25-$(OH)_2$ D_3 can be done directly in some cells of the immune system such as macrophages. The macrophage, like kidney cells, contains the enzyme 1-alpha hydroxylase that is capable of transforming Vitamin D into its active form, calcitriol. Exposure of the macrophage to some pathogens induces the production of CP27B (step 1) (which allows 25 OHD to enter the mitochondria for transformation into its active form 1,25-$(OH)_2$ D_3), (step 2) as well as vitamin D receptor (VDR) (step 3), which by binding to 1,25-$(OH)_2$ D3 increases the production of cathelicidin. (step 4). The antimicrobial activity of vitamin D appears to be primarily dependent on the induction of cathelicidins, which perform numerous functions that enhance both innate and adaptive immunity; help improve the digestion process within the phagolysosome through a non-oxygen dependent mechanism (step 5),[46] that can promote pathogen clearance by inducing apoptosis of infected epithelial cells, and induce paracrine responses in monocytes and T and B lymphocytes, among others.[45] Vitamin D also has direct effects on other cells, for example, epithelial cells, eosinophils, mast cells as well as T and B lymphocytes. (step 6)[47]

lymphocytic blastogenesis, NK cell activity, and regulation of IL-1 and IL-2.[35]

Moreover, in the elderly, a sleep pattern with poor quality (self-reported) was related to increased levels of IL-6 and this association was not explained by other factors, such as depressive symptoms, stress or loneliness, but exclusively by sleep disturbance as an independent factor.[36]

As for the clinical evidence, an adequate sleep pattern also appears to be beneficial in modulating the adaptive immune response. Spiegel et al evaluated the effect of sleep deprivation on the antibody response to influenza vaccination on 25 volunteers. The control group (who slept 8 h per night) developed a rise in IgG antibody titer against influenza virus 10 days after vaccination twice as high compared to the sleep deprived group (who slept 4 h for 6 nights, but then were allowed to recover with several days of 12-h catch-up sleep). Antibody levels were determined once again after 21–30 days, this time showing no difference in antibody levels between the groups. This might be explained by the effect of normalization of the sleep pattern in the group initially subjected to wakefulness.[37]

Table 1 presents 2 studies evaluating the effect of sleep in volunteers with an experimentally induced rhinovirus common-cold.[38,39] Shorter sleep duration was associated with an increased risk of developing a common cold in a dose-dependent manner. These results support the concept that people with a normal sleep pattern (7–9 h a night in adults) could increase the effectiveness of the immune system in the context of responses to viral URTIs. Up to now there are no specific studies on the effect of sleep and the immune response to SARS-COV-2.

Mindfulness

Albeit limited, there is some evidence suggesting that enhancing general physical and mental health may reduce URTI prevalence and severity.[40]

Specifically, mindfulness meditation has been proposed as a practice progressively capable to reduce experienced stress and negative emotion through increased awareness of physical, emotional, and cognitive aspects and heightened sensitivity to bodily sensation, which may lead to a healthier mind-body response to stress, albeit with little quantitative expression.[41]

One interesting clinical randomized trial on 94 adults aged 50 years or older who underwent either a professionally guided standardized 8-week meditation-based program of mindfulness-based stress reduction (MBSR) with weekly 2-h group sessions and 45 min of daily at-home practice (N = 51) or placebo (n = 51), showed that incidence, duration, and global severity of URTIs were 33%, 43%, and 60% lower in the meditation group compared with controls (whereas not all comparisons reached statistical significance). Also, URTI-related work absenteeism was significantly lower for the meditation group ($P < 0.001$).[42]

Based on the above, a constant program of professionally guided meditation strategy could be beneficial to achieve an effective stress reduction and probably to reduce the burden of URTIs, mainly in older adults.

Supplementation

i. Vitamin D

The hypothesis that vitamin D use could reduce the risk of respiratory infections began from observational studies showing that the most vulnerable populations (older adults, individuals with comorbidities, malnutrition, etc), shared an increased risk of vitamin D deficiency and that in addition, the incidence of respiratory infections increased in winter season where vitamin D levels drop, secondary to a shorter time of sun exposure.[43]

The effects of vitamin D on the immune system have been well studied, including the induction of catelicidine and β-defensins, modulation of immune cells migration to the site of infection, direct antiviral effects such as disruption of the viral membrane interfering with glycoproteins necessary for viral replication downregulating viral receptors, modulation of TLRs, clearance of respiratory pathogens by inducing infected epithelial cell apoptosis, and selective suppression of pro-inflammatory cytokine production (TNF-α, IFN-β, IL-8, IL-6, chemokine (C–C motif) ligand 5 [CCL-5]), among many others.[44,45] See Fig. 2.[45-47]

Although the biological basis of vitamin D intervention is clear, and multiple studies have described the benefits of vitamin D supplementation to reduce the risk of respiratory infections[48] and even to reduce intrahospital stays in mechanically ventilated patients,[49] the published study results so far have not been conclusive.

Of the 6 meta-analysis reviewed, only 2 agree on the benefit of vitamin D supplementation for decreased respiratory infections.[50,51] Subgroup analysis shows that the most consistent benefits are with daily or weekly supplementation[50–52] vs monthly supplementation, in patients with basal serum vitamin D < 25 nmol/L[51] and in asthma patients to reduce the risk of URTI-induced asthma exacerbations;[53] however, the high heterogeneity of the studies, the bias of having not measured the basal serum levels in most studies and the difficulty of establishing the diagnosis of outcome in a reliable way makes it difficult the attribution of real benefits and therefore the general application of their results.

An important point to be highlighted is the low level of adverse effects reported despite the administration of high doses of vitamin D, so the intervention could be considered relatively safe.[54]

Finally, the most important fact in considering supplementation in the context of COVID-19 would be to identify subjects with vitamin deficiency who would likely benefit most from supplementation.[55,56] At the moment, there is no universal consensus on ideal vitamin D levels,[57] and even less, the values that will permit to achieve a benefit on viral infections. Nonetheless, 40–50 ng/mL could be considered an appropriate value in most scenarios.[48]

ii. Vitamin C

The antioxidant properties of Vitamin C might influence the regulatory function of the immune system.[58] These effects have been observed in *in vivo* murine models, showing an increase in the production of IFN-α and -β that up-regulate

Dietary Supplementation	Potential Effects in COVID-19 infection	Main Natural Sources
Vitamin C	- Reduces inflammation and immune response, blocking nuclear NF-kß translocation. - Increase in IFN α and β production - Increase of hydrogen peroxide and other free radicals production.	Oranges, lemons, mangoes.
Flavonoids	- Reduces inflammation and immune response, blocking nuclear NF-kß translocation. - inhibit the $3CL^{pro}$ which would inhibit viral replication	Red wine, oranges, red fruits and vegetables.
Omega 3 and Fatty acids	- Blockage of pro-inflammatory cytokines like TNFα, IL-2 and IL6	Seafood (salmon, tuna), Nuts and seeds, plant oils.
Polyphenol	- Reduces inflammation and immune response, blocking nuclear NF-kß translocation.	Green tea, broccoli, apples.

Table 2. Possibly potentiating defense against COVID-19 of some vitamins and micronutrients and their main natural sources. *Modified from: Husson MO et al. Modulation of host defense against bacterial and viral infections by omega-3 polyunsaturated fatty acids. J Infect 2016; 73; 523–35.*[66]

immune regulatory responses.[55,59] Also, an *in vitro* study provided information that the addition of vitamin C to iron and/or cupper was related to an increase in the production of hydrogen peroxide and other free radicals, thus conferring antiviral properties to vitamin C.[58] Because of the previously mentioned effects, it has been proposed that vitamin C could have a protective effect against viruses in humans. A systematic review with Cochrane collaboration failed to demonstrate that vitamin C supplementation with doses over 200 mg reduced the incidence of colds in the general population, but the disease duration was reduced by 8% in adults and 14% in children.[60] Another study did not find a conclusive effect on prevention of COVID-19 with high doses of vitamin C.[55] Despite the inconclusive results with vitamin C prophylaxis for COVID-19, some authors recommend its use with the premise of the low risk of given intravenous vitamin C or through the oral route, added to the theoretical benefit in case of a serious infectious disease.[58,61–64]

ii. Omega 3 and adiponectin

Animal studies have shown that polyunsaturated fatty acids (PUFAs) with omega-3 could improve adiponectin plasma levels,[64] increasing the possibility of a regulatory immunologic response improvement, through blockage of pro-inflammatory cytokines like TNF-α, IL-2 and IL-6. This might be of particular interest in COVID-19 as one of the pathophysiologic mechanisms clearly demonstrated is inflammation of the pulmonary vascular endothelium, accompanied by increased angiogenesis.[65] An appropriate ingestion of PUFAs and omega-3 at doses of 250–500 mg/day could have a positive effect on the response to viral infections through messenger RNA export blockage of intracellular pathogens.[64,66] To date, there is no evidence of PUFA supplementation in prophylaxis of URTIs.

iv. Flavonoids

Among polyphenols epigallo-catechin-3 gallate is a flavonoid present in green tea, that blocked TNF-β translocation, in mice models of COVID-19 attenuating lung injury.[64,67] Another option is the use of traditional Chinese herbs, many of which contain cinanserin, quercetin, and herbacetin, among other substances. These have been shown to inhibit the coronavirus main proteinase ($3CL^{pro}$) and thus inhibit viral replication in SARS-CoV.[68–70] Glycyrrhizin acid derived from licorice root with a binding capability to ACE2 in

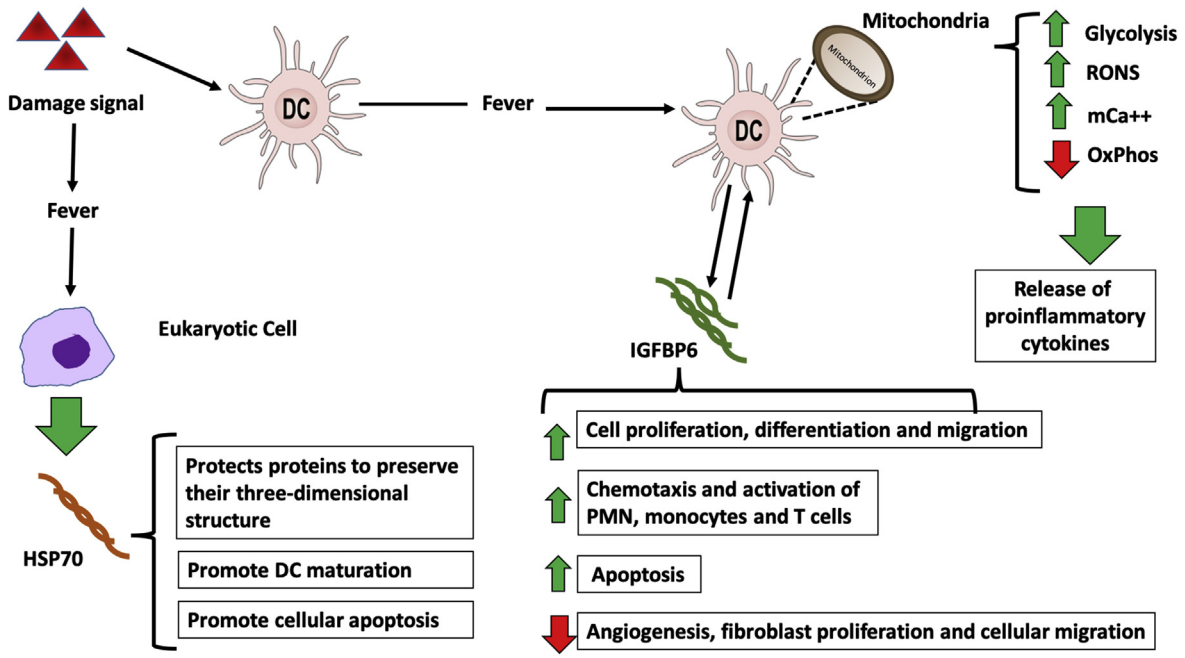

Fig. 3 In DCs, derived from human monocytes, subjected to temperatures >38 °C, a decrease in mitochondrial metabolism has been seen inducing a decrease in oxidative phosphorylation (OxPhos) and an increase in reactive oxygen and nitrogen species (ROS/RNS), intramitochondrial calcium and glycolysis. All this produces the release of proinflammatory cytokines that activate T lymphocytes. Fever favors the production of heat shock proteins (HSP) by all eukaryotic cells. DCs release insulin-growth-factor-binding-protein (IGFBP6) that has stimulating, but also self-regulating effects

combination with vitamin C has been used for SARS-CoV-2 pneumonia.[61,71]

The previously mentioned interventions are potential options that could improve the immune system against SARS- CoV-2, but more evidence is required.[70] See Table 2.

v. Zinc

Zinc is an essential trace element for the proper functioning of multiple biological processes within the human body, and its contribution to immunity is not an exception.[72]

The complete mechanism by which zinc could decrease the number or severity of viral infectious processes in general and of COVID-19 in particular is not exactly understood yet; however, effects have been observed on the binding of the viral agent to the mucosa and on its replication, as well as on the regulation of the inflammatory process;[73] enhanced benefits have been hypothesized when co-administered with other medications such as (hydroxy)chloroquine that could function as an ionophore, facilitating the entrance of zinc into the cells.[74] The human body's ability to store zinc is known to be low; its deficiency compromises the immune system, as has been evidenced occasionally by thymic atrophy, lymphopenia, and altered lymphocyte responses.[75] Its supplementation may reduce the risk of infectious processes, especially URTI, pneumonia, and diarrhea in both children and older adults.[76,77]

In relation to the clinical evidence, all of the 4 reviewed meta-analyses reported beneficial effects of zinc supplementation in children for the prevention of pneumonia[76,78-80] (especially for objectively diagnosed disease),[79] continuous administration vs short courses,[78] and with doses above 75 mg/day.[80]

Adverse effects are considered mild and include nausea and headache. However, it is important to avoid over-administration due to the risk of copper deficiency.[80]

NON-SPECIFIC IMMUNE STIMULANTS

Fever and hyperthermia

Fever is a complex autonomic response of vertebrates generated in response to endogenous

damage secondary to an infection, which has evolved over 600 million years and has been associated with increased survival and resolution of an infection.[81,82] It is a defensive response capable of improving innate and probably also the adaptive immune response, due to its effect on the DCs, whose functioning depends on body temperature, Fig. 3.[83,84]

Temperature rise from 39 to 40° Celsius (°C) increases the production of IL-6, IL-12, TNFα, IL-1β, IFNs, and prostaglandins (endogenous pyrogens) by various cells, while macrophage production of IL-10 and TNFα is reduced, driving the T helper (Th)-cell response towards Th1. Temperature restitution does not completely reverse these effects.[85] Endogenous pyrogens act on the thermoregulatory center in the hypothalamus, resulting in physiological responses that increase temperature.[83] They stimulate the expression of L-selectin, which favors leukocyte migration.[83] However, autonomic central nervous system (CNS)-derived norepinephrine has an opposite effect.[86] On the other hand, unbeneficial heat stress can cause the muscle to produce IL-6 and other myokines.[87]

Volunteers have been subjected to 39.5 °C baths, to experimentally resemble fever, while monocyte activation molecules were measured. CD14, CD11b (complement receptor) and of CD62L are upregulated, the latter enhancing leukocyte adherence to the vascular endothelium. If exposed to endotoxin, they release a large amount of TNF-α.[82,88] Feverish states also activate T lymphocytes with an increase in protein kinase C (PKC) and opening of thermolabile calcium channels (TRPV), increasing their cytotoxic activity.[89]

Thermal stress favors the release of acute phase reactants (C reactive protein, serum amyloid A, procalcitonin, complement C3, haptoglobin) and components of the coagulation cascade (fibrinogen and fibronectin A). These endogenous factors are related to the interaction with toll-like receptors (TLRs).[90]

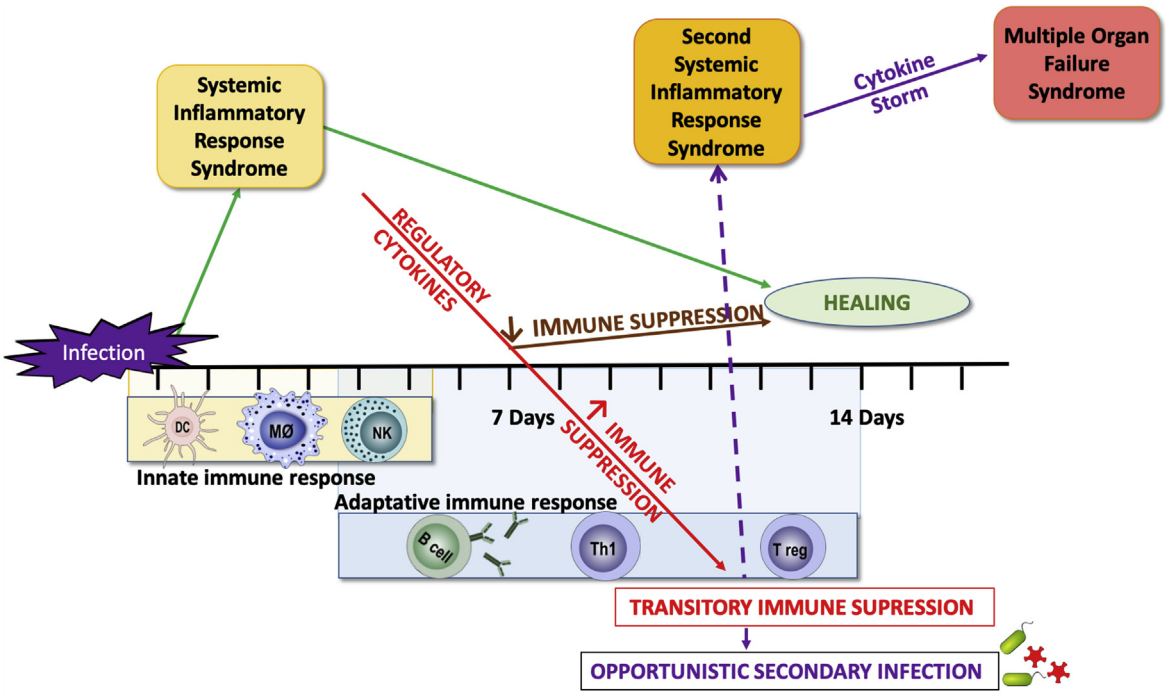

Fig. 4 The response to early damage has been clinically defined as systemic inflammatory response syndrome (SIRS) in which innate immune system cells and proinflammatory cytokines are involved; secondarily, the adaptive immune response also gets activated, allowing the host to heal, as long as a negative feedback is produced by regulatory cytokines to counteract the inflammation and limit the immune damage, generating a "temporary immunosuppression". However, if at this moment an opportunistic infection occurs or the inflammatory stimulus persists, a second SIRS can be generated that favors the massive release of inflammatory cytokines (cytokine storm) that can cause death due to the development of the multi-organ dysfunction syndrome (MODS)

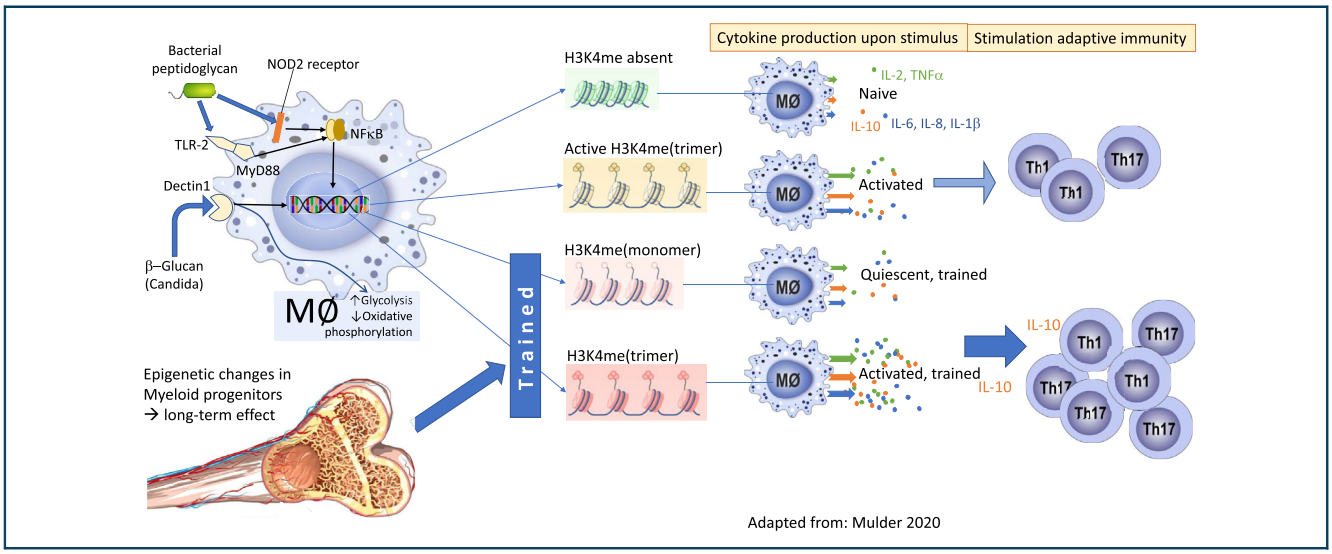

Fig. 5 In murine and *ex vivo* human experiments monocyte-derived DCs, upon stimulation with MV130 (Bactek), produced IL-12p70 and TNF-a enhancing Th1 proliferation, and IL-6, IL-1β, IL-8 ie, stimulating the development of Th17 cells. MV130 effects were shown to be generated after TLR activation and the serine/threonine-protein kinase-2 receptor interaction (RIPK2) and myeloid-88 (MyD88) pathways under control of IL-10.[104] Trained immunity effects have been shown to persist for months, as epigenetic changes also occur in the progenitor cells in the bone marrow[100]

Some investigators are evaluating whether the controlled induction of hyperthermia could have any therapeutic use in the control of infectious processes.[82] In the 5th century B.C., Hippocrates described the calming effect of malaria fever in epileptics. In the 19th century heat-therapy was used in the treatment of psychiatric disorders, and an Austrian psychiatrist received the 1927 Nobel Prize for his work on fever therapy in the treatment of neurosyphilis.[91]

However, hyperthermia also has anti-inflammatory properties. Brunt et al showed that serum, collected from volunteers submitted to 8-week heat-therapy (4–5 times/week sufficient to maintain rectal body temperature 38.5 °C for 60 min) upregulates HSP and anti-inflammatory and anti-oxidative proteins, thus augmenting the resistance of endothelial cells against inflammatory and oxidative stress. This suggests heat therapy might be a promising intervention to reduce cellular damage following ischemic events.[92]

The temperature rise during an infection improves the conditions for the systemic inflammatory response syndrome (SIRS) to develop more effectively,[88] Fig. 4. However, temperatures above 42 °C for more than an hour are no longer beneficial, as they produce a decreased recruitment and activity of NK cells, generate CNS damage and protein denaturation.[84]

As for clinical data, in the elderly with community-acquired pneumonia, mortality was significantly lower in patients who developed a febrile response (4 versus 29%).[93] Survival of infected patients admitted to the ICU was enhanced in those presenting 37.5–40 °C.[94] Also, pathogens replicate better at 37 °C than at higher temperatures.[95] Finally, during the 1918 Flu pandemic hydrotherapy with hot wrappings on chest and abdomen, soaked in boiling water thrice daily with the patient wrapped in a blanket resulted in 300% increased survival rate.[96]

Even so, the discussion whether fever is beneficial is still ongoing, as in most clinical trials withholding antipyretics increased survival, but not in all.[97,98] Definitely temperatures above 40 °C are no longer favorable. At population level, hypothetic models of seasonal influenza also showed diminishing fever with antipyretics seemed to increased mortality by 5%.[99] Surely enough, milder infections can have an afebrile course and even so a good outcome. Metanalyses have been inconclusive so far.[97,98]

Bacterial vaccines

Trained immunity refers to immunological memory of the innate immune system, thus being non-specific by definition. It enables a stronger immune response to a second stimulus.[100] A bacterial vaccine (BV) contains bacterial lysates or whole, live attenuated or dead cells of one or several pathogens. BV can be administered sublingually, orally or subcutaneously. In order to induce trained immunity the following characteristics are needed:

I. Contain trained immunity inducers, like pathogen and damage-associated molecular patterns (PAMPs and DAMPs) in addition to pathogen specific antigens.

II. Demonstrate effectiveness against heterologous pathogens, apart from the specific pathogens, targeted by the BV

Interestingly, by activating the innate cells to release IL-1β, IL-12 and TNFα BV also simulates the adaptive immune response.

The best-known BVs are Bacillus Calmette-Guérin (BCG), *Candida* and mixes of respiratory bacteria. Anecdotal evidence exists from the 19th century onward, but since the past century its scientific bases were settled. BVs have been used as adjuvants not only for reducing RTI, but also in allergy, asthma, and cancer treatment. The observation that monocytes from patients who were vaccinated with BCG responded vigorously to stimulation with unrelated germs (*Candida albicans*, *Schistosoma mansoni*, or influenza virus)[101] and epidemiologic reports that showed nonspecific protection in infants who were previously vaccinated with BCG, gave rise to the creation of the concept of "trained immunity",[102,103] (see section on BCG).

Nowadays, investigators have shown that trained immunity is based on epigenetic reprogramming of myeloid cells (dendritic cells, macrophages, NK-cells) that result in changes in their phenotypic and metabolic behavior. Consequently, they produce a stronger response to subsequent stimuli, since deoxyribonucleic acid sequences coding for proinflammatory cytokines and chemokines are partly kept open by histone methylation,[100] (see Fig. 5). Signaling is dependent on dectin-1, TLRs, and nucleotide-binding oligomerization domain-containing protein 2 (NOD2), depending on the trained immunity inducer being BCG, bacteria, or candida, respectively. In murine and *ex vivo* human experiments monocyte-derived DCs, upon stimulation with MV130 (a sublingual poly-bacterial vaccine), produced IL-12-p70 and TNF-α enhancing Th1 proliferation, and IL-6, IL-1β, IL-8 stimulating the development of Th17 cells through the receptor interacting serine/threonine kinase 2 (RIPK2) and myeloid differentiation primary response 88 (MyD88) coding gene pathways; conversely, a rise in IL-10 simultaneously balanced the immune-regulation.[104,105] Long-lasting effects, up to 12 months, have been documented as trained immunity also influences myeloid progenitor cells in the bone marrow.[106]

As for clinical evidence, a metanalysis of randomized trials demonstrated its efficacy as a preventive agent during the 1918 Spanish flu pandemic, reducing case fatality up to 70% in those subjects who received the bacterial vaccine compared to controls.[107] More recently, a prospective open pilot study of 17 subjects, six months' daily sublingual administration of a heat-inactivated, respiratory BV, MV130 (including *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Moraxella catarrhalis* and *Hemophilus influenzae*, respectively 60-15-15-4-3-3%, respectively), significantly reduced the rate of recurrent RTIs.[108] Similarly, OM85, a lyophilized alkaline extract of mixed respiratory pathogens reduced lower respiratory tract infections in high-risk infants from 65 to 42% the first year.[109] Recently, investigators demonstrated that virus can also enhance trained immunity in cord-blood immune cells of newborn infants of HBV-infected mothers, enabling them to respond vigorously to a bacterial challenge.[110]

In conclusion, education of the innate immune response by the administration of multipurpose BV produces an intense pro-inflammatory response during subsequent heterogeneous infections (viral or bacterial) while maintaining immune system homeostasis.

M = metanalysis, D = DBPC, R = Randomized with open control, O = observational.

Inter-vention	Design				A (active) P(placebo)	Variable	Outcome**			Comments and sub-group analyses		Reference
							B	U	N B			
Exercise	M	D	R	O 1	Physical activity (PA) High: n=287 Low: n=787	IRR for URTIs	☺			URTI symptoms were self-reported. High levels of PA (equivalent to jogging) were associated with 18% reduced risk (IRR=0.82, 95% IC=0.69-0.98) vs. low levels of PA. IRR for URTI decreased with increasing age for both sexes		Fondell 2011(10)
Forest bathing	M	D	R	O 3	A = 12 Self-controls	NK cell activity	☺			Individuals were their own controls: first trip to town, months later trip to forest. NK cell activation confirmed in last trial with inhalation phytoncides during night rest in town.		Li 2008(28) Li 2009(29)
						NK cell number	☺					
						Granzimes	☺					
Sleep	M	D	R	O	A = 164 P = 0	Common cold and virus-specific neutralizing antibody titers	☺			Experimental infection with rhinovirus: shorter sleep duration prior to viral exposure was associated with increased susceptibility to the common cold and lower IgG against influenza.		Prather(39)
				O	A = 153 P = 0		☺			In experimental infection with rhinovirus preceding poorer sleep quality and duration <7 h were associated with lower resistance to illness		Cohen(38)
				O	A = 27 P = 0	IL-12 producing DC precursors	☺			In an experimental setting sleep can effectively enhance adaptive immune responses (DC precursors producers of IL-12)		Dimitrov(31)
				O	A = 11 C = 14	IgG levels	☺			Case-control: Sleep deprivation decreased flu antibodies		Spiegel(37)
				O	A = 83 P = 0	IL 6 and others (quality sleep)	☺			Older adults vulnerable to effects of sleep disturbance due to age and inflammatory regulation.		Heffner(32)
Meditation/mindfulness	M	D	R	O	A= 51 C = 51	Prevention of URTIs	☺			Training in meditation may be effective in reducing ARI illness burden in adults >50 years.		Barrett 2012(42)
Vitamin D	M	D	R	O	A= 2945 P= 2897	RTI	☺			No difference between healthy subjects vs patients, children vs adults, vit. D sufficient vs deficient		Bergman 2013(50)
										Secondary outcomes		
										Beneficial	Not Beneficial	
										RTI Daily dose	RTI bolus	
	M	D	R	O	A= 2440 P= 2387	RTI in infants (1.75-18 months)			☹	Dosing: 300 to 6800 IU/day None of the covariates (age, dosing, regimen of Vit D, study length) any significant influence on the RR		Mao 2013(152)
	M	D	R	O	A = 6503 < 18 y	RTI			☹	Secondary outcomes		Xiao 2015(53)
										Beneficial	No beneficial	
						Acute asthma related to RTI	☺			Prevention of influenza	Prevention pneumonia All-cause mortality Rate of hospital admission due to RTI	
						Recurrent pneumonia			☹			
	M	D	R	O	A = 3198 P =	Incidence rate of pneumonia in < 5 yo			☹	Secondary outcome		Yakoob 2016(153)
										Beneficial	No beneficial	
											. Mortality due to . pneumonia . All-cause mortality . Any Hosp. admission	

	M	D	R	O	A = 7053 P =	RTI			☹	Average daily dose was 1500 IU (300 to 3700 IU) Secondary outcomes Beneficial: RTIs among those with daily/weekly vitamin D supplementation No beneficial: Risk of a first laboratory-confirmed viral RTI; Absence duty or school; Severity RTI symptoms; RTI among those with low (20 ng/ml) Vit D; RTIs among those with bolus administration	Vuichard 2016(52)
	M	D	R	O	A = 11,321 P =	RTI	☺			Subgroup analysis: protective effects with daily or weekly vitamin D, not with bolus doses. Secondary outcomes related to ARTI Beneficial: . ARTI with baseline . 25(OH)D <25nmol/L . ARTI 1.1-1.5.9 years . ARTI with BMI <25 . ARTI in asthmatic and not asthmatic patients Not beneficial: Upper // Lower RTI; Hospital admission or emergency department; Use of antimicrobials; Work or school absence; Death from RTI; Death all-cause; Death (any infection)	Martineau 2017(51)
Vitamin C	M	D	R	O	A = 11306 P = not stated	Incidence of colds			☹	Dosing > 0.2g	Hemila 2013(60)
						Disease duration	☺				
Vitamin C	M	D	R	O	A=160 P=160	Decrease morbidity in patients with sepsis-related ARDS	☺			Mega-dosing: 15g/d IV	Fowler 2019 Carr 2020 (154, 155)
Zinc	M	D	R	O	A= 818 P= 902	Pneumonia	☺			Children <5 years old The range of zinc doses (0.7 to 1.8 mg zinc/kg), no significant correlation between dose and effect size for pneumonia incidence	Bhutta 1999(78)
	M	D	R	O	A=3819 P= 3840	Respiratory tract infections	☺			Zinc supplementation for 3 months for children <5 years Secondary outcomes Beneficial: Lower respiratory tract infection or pneumonia No beneficial: Days with respiratory illness	Aggarwal 2007{ Aggarwal 2007}
	M	D	R	O	<5 years N= 49,450	Acute lower RTI (well defined criteria)	☺			Incidence of lower RTI as defined by specific clinical criteria. No effect mean baseline age, geographic location, nutritional status or zinc dose.	Roth 2010(79)
						Acute lower RTI definitions based on caregiver report			☹		
	M	D	R	O	N=1402	Duration of common cold dose >75mg/d	☺			Zinc Lozenges	Hemila 2011(80)
						Duration of common cold dose <75mg/d			☹		

Other suppl	M	D	R	O	A = 143 ICU patients P = 129	-MV-free day	☺			Active = EPA + GLA by 7 days (enteral supplementation of n-3 fatty acids, γ-linolenic acid) EPA: Eicosapentaenoic acid (an omega-3 fatty acid) GLA: gamma linoleic acid MV: mechanical ventilation ICU: intensive care unit	Rice 2011(156)
						-ICU-free days	☺				
						-Organ failure-free days (Non-pulmonary) -Stopped for futility	☺				
Fever/ Hyper-thermia	M	D	R	O	6 trials	Mortality risk of antipyretic medication use in critically ill patients with infection		😠		The 6 included RCT we heterogenous in design, study-population and intervention. Most not primarily designed to detect relation fever control – mortality risk. $I^2 = 34.9\%$. Odd ratios for mortality 0.96 (fixed) /1.01 (random effect)	Jefferies 2011(98)
	M	D	R	O	Critically ill A = 38, permit fever <40℃ C = 44, treat aggressively	Mortality in permissive group versus aggressively treating with paracetamol.	☺			Study was stopped preliminarily, due to mortality of 7 (aggressive treatment) versus 1 (permissive group).	Schulman 2005(94)
	M	D	R	O	A = 12 Self-controls	TNFα , LPS	☺			Individuals immersed in a hot water bath (39.5℃) for 2 hours, Blood samples were taken before and when body temperature reached its highest level;	Zellner(88)
						CD14, CD11b	☺				
					one week later	CD114	☺			hyperthermia induces CD14 and CD11b expression in monocytes and this expression enhances their production of TNF-α	
Bacterial vaccine	M	D	R	O	Subjects with influenza A = 20,087 P = 166,870	Preventing pneumonia	☺			Random effect model Preventing pneumonia: RR 0.66 (95% CI, 0.53–0.81). Case fatality: RR 0.58 (95% CI, 0.41–0.82)	Chien 2010(107)
						Reducing case fatality	☺				
						Subgroup: 3 military studies	☺			Military (less bias): Fixed effect model: case fatality RR 0.30 (0.18-0.50)	
						Incidence of influenza A	☺			Reduced in 9/13 trials vaccinated versus non vaccinated subjects; metanalyses above focused only on all subjects **with influenza**: vaccinated versus non-vaccinated)	
	M	D	R	O	A = 17 MV130# for 6months	RRTI	☺			Patient's rate of RRTIs reduced compared with 1 year prior to initiation of therapy ($P < 0{\cdot}0001$).	Alecsandru 2011(108)
Probiotics	M	D	R	O	A = 3720 (12 trials) P or controls	% subjects with acute URTI	☺			**Population** People of all ages **Types of interventions** A: Any probiotic (single/mixture of strains, any dosage regimen/administration route) > 7 days-3 months, versus placebo/no treatment. **Secondary outcomes** 1. Time off from childcare center, school or work (a proxy of severity of disease).	Hao(119)
						Rate ratio of episodes of acute URTI	☺				
						Mean duration of an acute URTI episode	☺				

DLE	M	D	R	O	Sepsis in children in Intensive Care Unit: DLE = 15 Control = 108	CRP (p 0.04) Total lymphocyte/ neutrophil count (p=0.0005/ 0.007) ESR		😐		**Design** A retrospective electronic chart review of in-patients. **Types of interventions** DLE during time un this unit and evaluations after 72h. **Outcome** Sepsis severity markers in blood (CRP, TLC, etc.)	Castrejon Vazquez 2019(129)
					A: local surgery =17 B: DLE=18 C: Cryo+DLE = 19 Healthy controls=20 TOTAL: A = 37 P = 39	Presence of cervical lesion at 24 months	🙂			54 women with cervical cancer: all had low-grade intraepithelial squamous lesions. Group C: recurrent. DLE orally 5 weeks and locally injected 5x in 2 weeks. DLE repeated at 3, 6, 9, 12 months according to evolution. **Outcomes:** - Presence of cervical lesions less frequent in gpB (p<0.05) - Viral load: only obtained in subgroup, NS differences - No consistent immunologic changes in DLE groups (B-C) ▪ Group B, but not C, rise in %CD3+ IFN-γ+ ▪ Group C: from start to finish elevated %CD3+IL-10+ Some clinical data seem promising, but not consistent. Lacking methodological rigor. This makes us consider that use is reserved at the discretion of the physician	Rodriguez Flores 2015(126)
						Viral load		😐			
						Immunologic - rise in %CD3+ IFN-γ+ - %CD3+IL-10+		😐			
Pidotimo	M	D	R	O	A = 4344	Frequency of	🙂			Good efficacy and safety in treatment of	Niu 2019(138)
d	l					RTIs				pediatric RTIs.	
BCG	M	D	R	O	A = 2,083 P = 2,089 BCG-Denmark Low Weight neonates (<2,500 g) Ginea-Bissau	Neonatal Mortality Rate Ratio (MRR)	🙂			Active = early vaccination Control = normal according to local policy • Total: -30% (0.70; 95% CI; .47-1.04) • For infectious diseases: -43% (0.57; 95% CI; .35-.93) • For non-infectious diseases: NS	Biering-Sørensen 2017(142)
	M	D	R	O	n = 6,544 BCG-Denmark Low Weight neonates (<2,500 g)	Mortality Rate Ratio (MRR)	🙂			• Day 3: -55% (0.45; 95% CI, .32-.93) • Neonatal: -38% (0.62; 95% CI, .46-.83) • 12 months: -16% (0.84; 95% CI, .70-1.00)	Biering-Sørensen 2017(142)
Hetero-loguous vaccines	M	D	R	O	Clin trials = 17,190 Observa-tional = 101,123	Association ↓ all cause 2-5yo mortality with measles containing vaccines (MCV)	🙂			Receipt of standard titer MCV associated with ↓ in all cause childhood mortality: - 4 clinical trials: RR 0.74 (0.51 to 1.07) - 18 observational studies: RR 0.51 (0.42 to 0.63) high risk of bias - effect seemed stronger in girls than in boys	Higgins 2016(157)
	M	D	R	O	Mice: A = 4 P = 3	Live Attenuated Influenza	🙂			Increase in TLR3/7 signaling pathways are necessary for the observed immediate and short-term protection	Lee 2018(151)
						Vaccine, cross-protection against RSV					

* 🙂 = beneficial, 😐 = unclear if any effect, ☹ = No beneficial outcome
# MV130: a bacterial vaccine composed of heat-killed respiratory bacteria.
M = metanalysis, D = Double blind placebo controlled trial, R = Randomized trial with open controls, O = observational
BCG: Bacillus Calmette-Guérin; BMI = body mass index; DLE: Dialyzable leukocyte extract; EPA: Eicosapentaenoic acid (an omega-3 fatty acid); GLA: gamma linoleic acid; I^2: Heterogeneity; IgG: Immunoglobulin G; ICU: intensive care unit; IU: International units; IRR: Incidence rate ratio; LPS: Lipopolysaccharide; MCV: measles containing vaccines; MRR: Mortality Rate Ratio; MV: mechanical ventilation; OR: Odds ratio; RR = relative risk; RSV = respiratory syncytial virus; (U)RTI = (Upper) respiratory tract infection; TNF-α: Tumor necrosis factor alpha.

Table 3. Overall highest level of evidence for each intervention.[152-157] BCG: Bacillus Calmette-Guérin; BMI = body mass index; DLE: Dialyzable leukocyte extract; EPA: Eicosapentaenoic acid (an omega-3 fatty acid); GLA: gamma linoleic acid; I2: Heterogeneity; IgG: Immunoglobulin G; ICU: intensive care unit; IU: International units; IRR: Incidence rate ratio; LPS: Lipopolysaccharide; MCV: measles containing vaccines; MRR: Mortality Rate Ratio; MV: mechanical ventilation; OR: Odds ratio; RR = relative risk; RSV = respiratory syncytial virus; (U)RTI = (Upper) respiratory tract infection; TNF-: Tumor necrosis factor alpha

Intervention	Conclusion
• Exercise	Regular exercise has a probable immunoprotective and immunoregulatory effect. Older adults may obtain the greatest exercise-induced benefits for their immunological health.
• Forest bathing	Forest walks might be beneficial to boost innate immunity, particularly in times of a pandemic; evidence is limited, but there are no direct health-related safety issues.
• Sleep	The potential benefit of adequate sleep patterns overweighs the low level of evidence available up to date
- Mindfulness	A constant program of a professionally guided meditation strategy could be beneficial to achieve an effective stress reduction and probably to reduce the burden of URTIs, mainly in older adults
• Vitamin D	The published study results so far have not been conclusive. Subjects with vitamin deficiency would likely benefit most from supplementation. The intervention could be considered relatively safe
• Vitamin C	A Cochrane collaboration systematic review showed daily doses of 0.2 mg reduced the duration of common cold. In ARDS patients very high IV doses might be beneficial. More data are needed.
• Zinc	All 4 meta-analyzes have reported beneficial effects of zinc supplementation in children for the prevention of pneumonia. Important to restore the redox balance. Adverse effects are considered mild
• Other supplements	Several have anti-inflammatory potency, but there is no evidence of PUFA supplementation in prophylaxis of URTIs. Polyphenols display zinc ionophore capacity.
• Fever and hyperthermia	Fever during infection enhances survival of living beings. Solid evidence in human beings still lacking. Positive effects unless temperature exceeds 40°C for prolonged periods of time.
• Bacterial vaccine	Reduced mortality according to metanalysis of trials conducted during the 1918–19 influenza pandemic. Mechanisms are progressively better understood.
• Probiotics	A modest efficacy of some probiotics (Lactobacillus and Bifidobacterium strains) was found in reducing the incidence and duration of viral respiratory infections
• DLE	Though it might seem of benefit, evidence of the reduction of infections is very scarce and quality trials are lacking.
• Pidotimod	With the use of Pidotimod there is a lower recurrence of respiratory tract infections as compared to conventional treatment and less use of antibiotics
• BCG	We encourage to wait for the results of the ongoing RCT before using BCG vaccination for the prevention of COVID-19
• Hetero-loguous vaccines	The immune stimulation produced by heterologous vaccines enhances the response to other pathogens, different from the one in the vaccine and was associated with reduced all-cause infant mortality (e.g. measles vaccine, BCG).

Table 4. Concluding remarks per intervention in bullet-point format. *BCG = Bacillus Calmette-Guérin; DLE = Dialyzable leukocyte extract; PUFA = Polyunsaturated fatty acid; RCT = randomized controlled trials; URTIs = Upper-respiratory tract infection*

Probiotics

Probiotics are "live microorganisms that, when administered in adequate amounts, confer a healthy benefit to the host".[111] Administered orally, they activate TLR-2 and TLR-3 in intestinal epithelial cells, with subsequent increased production of IL-6 and other cytokines, chemoattractant for type I macrophages. This thus increases the number of macrophages and dendritic cells in the intestinal lamina propria, with greater production of TNF-α and IFN-γ that favor a state of tolerance with increased immunoglobulin (Ig)A+ cells.[111-113] Cytokines produced locally in the intestine can influence the activity of immune cells at distant sites, including the bronchi, inducing the activation of regulatory T cells that release IL-10. Additionally, probiotics strengthen the intestinal barrier by increasing mucin and tight-junction proteins, as well as goblet and Paneth cells that produce bactericidal substances.[111-113]

Modulation of the gut microbiota is a mechanism by which probiotics maintain the balance and suppression of harmful pathogens. The viability of probiotics is crucial for the interaction with epithelial cells and macrophages, mainly favoring the innate immune response, with increased microbicidal activity of peritoneal and splenic macrophages.[112] Probiotics protect against intestinal colonization of pathogens by direct induction of their death, by competition for nutrients and by activation of the mucosal-associated immune system. They also protect against some remote infections. In a study in human immunodeficiency virus (HIV)-positive patients, oral administration of probiotics was associated with an increase in type I and II interferons (particularly IFN-α) in intestinal cells and peripheral mononuclear cells, with an antiviral effect related to decreased viral spread.[114] In a clinical trial in China, oral administration of probiotics was associated with increased serum and secretory IgA and IFN-γ after 12 weeks of treatment, concluding that probiotics are safe and effective in reducing episodes of the common cold. Likewise, the potentiating effect on the production of antibodies or increased activity of NK cells of specific strains has been documented in elderly people[115,116]

An intercommunication between the intestine and the lung has been proposed in the pathogenesis of some respiratory conditions. This opens the possibility of the potential benefit of probiotics under such conditions. In 2 meta-analyses, a modest efficacy of some probiotics (*Lactobacillus* and *Bifidobacterium* strains) was found in reducing the incidence and duration of viral respiratory infections[55, 117] Jayawardena 2020.[118,119] The existence of intestinal dysbiosis has been reported in critically ill patients.[120] Two clinical trials found that mechanically ventilated patients receiving probiotics (*Lactobacillus rhamnosus*, *Bacillus subtilis*, and *Enterococcus faecalis*) developed ventilator-associated pneumonia less frequently compared to the control group (placebo).[117]

A small case series from China showed that some patients with COVID-19 had dysbiosis with decreased *Lactobacillus* and *Bifidobacterium,* and a link has been proposed between COVID-19 case fatality and diet, associating a diet rich in fermented vegetables –probably *Lactobacillius*-rich– reduced mortality.[121] Although it will be interesting to carry out studies to evaluate the efficacy of probiotics in the treatment of COVID-19, it is essential to know more about the pathophysiology of this disease before conducting clinical trials with probiotics in these patients.[117]

Dialyzable leukocyte extract ("transfer factor")

Dialyzable leukocyte extract (DLE) is a mixture of low-molecular-weight peptides (<10 kDa) that are released on disruption of peripheral blood leukocytes from healthy human subjects. It is used as a non-specific immune modulator. It originated from the historically controversial therapeutic resource defined as "Lawrence Transfer Factor", a blood product with the ability to passively transfer antigen-specific cell mediated immunity. In 1949, Lawrence published the successful transfer of late skin sensitivity to tuberculin (PPD) to PPD negative patients,[122] and in 1952 he successfully transferred sensitivity to partially purified preparations of the streptococcal substance M by the administration of suspensions of leukocyte components released by cell lysis by repeated freezing and thawing or distilled water lysis.

The further purified DLE, composed of only small peptides, caught the interest of a number of

researchers in demonstrating mechanisms, mediators and reproducing clinical results in different diseases. Among others, Myles et al aiming to identify the source of DLE's effects, performed experimental studies on murine and human individuals, and they found that the cell extract derived from TCD8+ antigen-specific memory lymphocytes induces the release of IL-6 by antigen-presenting cells (APCs) (DCs and myeloid-origin monocytes).[123] These cells also induce the release of IL-17 by naive $CD8^+$ T cells, without the need for contact with the APCs.[124]

Ramirez-Ramirez et al found in an *ex-vivo* study of cells from umbilical cord of new born infants and bone marrow of adults, that DLE increases the differentiation of $CD34^+$ toward a $CD56^+CD16^+CD11c+$ NK-like cell population endowed with properties such as IFN-y production, tumor cell cytotoxicity, and the capability of inducing γ-δ T lymphocyte proliferation, all together with an intense stimulation of the innate immune response.[125] In addition, DLE appears to have the ability to regulate cytokine production by PBMCs. In *in vitro* studies, Rodríguez-Flores found that T-cells from healthy individuals could produce TNF-α, IL-12 and IL-10 after DLE stimulation and thus defining monocytes as the main cellular population that produced TNF-α after stimulation with DLE and showing the direct activation of monocytes via TLR2.[126,127]

Based on the above cited and results of several other researchers, the exact content of DLE is being elucidated: it is assumed to possess oligoribonucleopeptids and fragments of the T-cell receptor;[123,128] these immunomodulating peptides stimulate the innate immune system by activating monocytes to produce IL-6 and IL-8, but also the adaptive immune system by enhancing the release of IL-2, IFN-γ and TNF-α from Th1 cells. Even so, it also enhances immune regulation as a rise in IL-10 has been documented.[126]

Clinically, it has shown benefit in some trials against infections,[126,129,130] cancer –both in murine and in human studies–[126,128,131]) and autoimmune diseases. Even so, searching PubMed indexed journals randomized trials and DBPC studies with DLE are still very scarce and thus its use remains poorly sustained at the moment, but it might be a promising option for the future.

Synthetic molecules (pidotimod)

Pidotimod (3-L-pyroglutamyl-L-thiaziolidine-4-carboxylic acid, CAS 121808-62-6) is a synthetic dipeptide with immunomodulatory properties, which is employed in the prevention and treatment of respiratory tract infections in children and adults.[132]

Early studies in humans demonstrated that pidotimod induces the bactericidal activity of alveolar macrophages,[133] while also activating innate immunity at NK cell level activity.[134] Recently, research has shown that monocyte adhesion and chemotaxis, as well as the migration of IL-2 activated T cells, induced by pidotimod requires the activation of the phosphatidylinositol 3-kinase pathway (PI3K/Akt) signaling pathway through the CXC chemokine receptor 3A (CXCR3A) isoform.[135]

Pidotimod is also capable of inducing the maturation of DCs and increasing the expression of major histocompatibility complex class II cell surface receptor (HLADR) and co-stimulatory molecules CD83 and CD86. Pidotimod also induces DC to produce pro-inflammatory molecules monocyte chemoattractant protein-1 (MCP-1) and TNF-α, which drive T cell proliferation and differentiation towards a Th1 phenotype.[136]

Furthermore, the use of pidotimod for community-acquired pneumonia treatment in adults has demonstrated that it upregulates the expression genes involved in inflammation and chemotaxis.[137]

As for the clinical evidence, a meta-analysis assessing the effectiveness and safety of pidotimod reviewed 29 randomized controlled trials including 4344 pediatric patients were included. While the meta-analysis questioned the quality of some of the trials under review, it concluded that pidotimod treatment resulted in a significant decrease in instances of recurrent RTIs (RR 1.59; 95% CI 1.45–1.74, $p < 0.00001$) in comparison to conventional treatment.[138]

SPECIFIC VACCINES WITH HETEROLOGOUS ACTIVITY

Bacillus Calmette-Guérin vaccine

The Bacillus Calmette–Guérin (BCG) vaccine has been used in humans since 1921 to prevent tuberculosis and other infections due to Mycobacterium tuberculosis (MT). Its administration is a public policy in many developing and some developed countries.

More than 130 million children per year are vaccinated with BCG. Its safety has been demonstrated even in preterm and/or low-birth-weight infants.[139] Infrequently, non-suppurating complications have been documented, be it of spontaneous resolution without treatment in 4 to 6 months. Less frequently seen suppurating complications, such as lymphadenitis abscess, might benefit from needle aspiration.[140] It is a live vaccine and thus contraindicated in immune deficient subjects.

Interestingly, the effects of BCG vaccination are not limited to the prevention of MT infections. It has several non-specific (also known as heterologous) effects. These include the prevention of other infections, its use for the treatment of cancer (eg, bladder cancer) and others. A retrospective study using data from the Official Spanish Registry of Hospitalizations (CMBD-HA) to identify differences in hospitalization rates (HR) in BCG-vaccinated children showed a risk reduction in hospitalizations for sepsis not attributable to tuberculosis in children under 1 year of age of 52.8% and for respiratory infections in <15 years of 41.4%.[141] A randomized controlled trial in Guinea-Bissau in low-birth-weight infants (<2500 grs), vaccinated with BCG at birth or at 6 weeks of life, as their routine practice, showed a reduction of neonatal mortality of 48%, of infant mortality of 21% and 41.4% for URTIs in <15 year-olds.[142] A randomized controlled trial in elderly (60–75 years old) who received BCG vaccinations as 3 monthly doses, showed a reduction of the incidence of acute URTIs.[143]

Furthermore, BCG has shown to induce a better response to other vaccines. In a double blind placebo controlled DBPC study, subjects who received BCG vaccination 14 days before the trivalent influenza vaccine by intramuscular route had a more pronounced increase and accelerated induction of functional antibodies against the influenza strains contained in the vaccine and a larger production of IFN-γ and IL-6 in response to unrelated pathogens.[144] In another study, subjects vaccinated with BCG who 28 days later received the yellow-fever live attenuated vaccine had a reduction of viremia that highly correlated with the upregulation of IL-1β, a heterologous cytokine associated to the induction of trained immunity or innate memory in monocytes. This has been validated through genetic, epigenetic and immunological studies, showing epigenetic reprogramming in monocytes resulting in a protection against non-related viral infection.[145]

During the COVID-19 pandemic, there seems to be a strong correlation between BCG index (the degree in which universal BCG vaccination is diploid in a country) and COVID-19 mortality: after correcting for many socioeconomic and pandemic-related confounders investigators found for every 10% increase in the BCG index a 10.4% reduction in COVID-19 mortality.[146] This association does not allow us to conclude anything about causality, however. Likewise, countries that have maintained the universal BCG vaccination policy have less morbidity and mortality per capita in some studies, but not in others.[147]

Nevertheless, the development of a specific vaccine for COVID-19 is urgently needed. Its inclusion as a worldwide public policy could take more than a year and be expensive. In the meantime, it is desirable to consider the BCG as an accessible, cheap, and safe alternative to possibly reduce the COVID-19 burden.[148] However, its efficacy to prevent SARS-CoV-2 infection or its severity has yet to be demonstrated in prospective randomized trials: up to now, there are 5 such clinical studies registered in Europe in high risk adult subjects. Four in healthcare professional who take care of infected subjects and 1 in subjects >60 years.(clinicaltrials.gov).

Other heterologous vaccines

Looking for other options to improve the response of our immune system against SARS-COV-2, one of the fields studied, is the immune response to vaccination. A clinical question is

whether there is any secondary, non-specific immune response to immunizations that could favor the response to other viruses, including SARS-COV-2. There is a concept called heterologous immunity, which consists of a response of the immune system to one or more pathogens, after exposure to a different infectious agent. This type of response can occur after previous exposure to a pathogen or through immunization.[149]

During the first years of life, the immune system is in an active state of learning and development. Therefore, the child's innate and cellular adaptive immune responses are in an increased state of alertness, influenced by frequent exposure to different pathogens, but also to many immunizations with viral and bacterial vaccines. This scientific basis could may be explain that certain vaccines might help to generate an immune response to a pathogen, different from the one applied.[150]

Apart from BCG, another of the vaccines studied in this pandemic is measles, which could have a positive effect when exposed to other pathogens, reducing mortality from infections not related to vaccination, including SARS-CoV-2. This beneficial effect could be mediated by increased Th1-type responses and activation of CD4 + T-cells, producers of IFN-γ, as well as CD8 memory cells.[150] The measles vaccine may have protective effects against unrelated infections, mainly in the airway and gastrointestinal tract.[149,150]

It has been proposed that influenza vaccination could also induce a heterologous response to other viral pathogens, such as respiratory syncytial virus, through the innate immune response, mediated by TLR3 and TLR7.[151, 158]

Despite the aforementioned, in the face of this pandemic it shall be important to continue the investigation and monitor whether heterologous vaccination might offer any additional protection. Hypothetically, it could be one of the factors explaining why children have been affected in a much lesser degree than adults, with a mortality rate 1:1000 compared to adults. However, studies are needed to know what role vaccines with a heterologous immune response could play at the moment a subject is exposed to SARS-CoV-2.

FINAL REMARKS

Without claiming to be complete, in this review article we presented a dozen of interventions that could possibly enhance the innate immune response, human's first-line defense when facing exposure to SARS-CoV-2. The interventions were divided into: 1) related to lifestyle, 2) actions with a non-specific booster effect on immunity, and 3) specific antigens causing a heterologous activation of the immune response. As for now, none of the interventions has proven effective for preventing COVID-19, though several are under investigation in clinical trials. However, there is evidence for almost all in the context of RTI and even directly in viral respiratory infections or severe RTI in the ICU; we tabulated what seemed to be studies with the best level of evidence for each intervention in Table 3. In the light of the threat for all of us of an imminent infection some interventions, even though evidence for their efficacy is not rock-solid yet, could be considered options that might help enhance the innate immune system and thus worthwhile, especially, as most lack the risk for serious adverse events. Together with the techniques focusing on reduction of the viral load, such as social distancing, equipment for personal protection and adequate hand hygiene, improving our first-line defense seems vital. Finally, we add in Table 4 bullet-points as a concluding summary, making it clear more direct evidence is needed in this important area.

Abbreviations

ACE2: Angiotensin converting enzime-2; APC: Antigen-presenting cell; BCG: Bacillus Calmette-Guérin; BV: Bacterial vaccine; CCL-5: Chemokine (C–C motif) ligand 5; CI: Confidence interval; CNS: Central nervous system; COVID-19: Coronavirus disease-2019; CXCR3A: CXC chemokine receptor 3A; DAMPs: Damage-associated molecular patterns; DC: Dendritic cell; DLE: Dialyzable leukocyte extract; $G\alpha_s$: G protein coupled receptor alfa-subunits: HSP; Heat shock proteins: HLA-DR; Major histocompatibility complex class II cell surface receptor: ICAM-1; Intercellular adhesion molecule type 1: IFN; Interferon: IG; Immunoglobulin: IGFBP6; Insulin-like growth-factor-binding-protein 6: IL; Interleukin: MBSR; Mindfulness-based stress reduction: mCa^{++}: Intramitochondrial calcium; MCP-1: Monocyte chemoattractant protein-1; MODS: Multi-organ dysfunction syndrome; MyD88: Myeloid differentiation primary response 88; NF-κB: Nuclear factor kappaB; NK: Natural killer; NOD2: Nucleotide-binding oligomerization domain-containing protein 2; OR: Odds ratio; OxPhos: Oxidative phosphorylation: PAMPs; Pathogen-associated molecular

patterns: PBMC; Peripheral blood mononuclear cell: PI3K/Akt: Phosphatidylinositol 3-kinase pathway; PKC: Protein kinase C; PPD: Purified protein derivative (tuberculin); PUFA: Polyunsaturated fatty acid; R_0: Basic reproduction number: REM; Rapid eye movement: RIPK2; Receptor iteracting serine/threonine kinase 2: RNA; Ribonucleic acid: RNS; Reactive nitrogen species: ROS; Reactive oxygen species: SARS-CoV-2; Severe acute respiratory syndrome coronavirus 2: SIRS; Systemic inflammatory response syndrome: TCR:T-cell receptor; Th: T helper-cell; TLR: Toll-like receptor; TNF-α: Tumor necrosis factor alpha; TRPV: Thermolabile calcium channels; URTI: Upper-respiratory tract infection

Author contributions

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