POTENTIAL USAGE OF IMMUNE-MODULATING SUPPLEMENTS OF THE *ECHINACEA* GENUS FOR COVID-19 INFECTION

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ABSTRACT The symptoms and progression of viral respiratory infections, such as the novel COVID-19 pandemic, is thought to be highly related to the host immune response. Populations with impaired immune response are particularly vulnerable to COVID-19; on the other hand, amplified immune response manifesting in "cytokine storm" is predicted to lead to adverse outcome. Consumption of immune-modulating supplements such as *Echinacea purpurea* is often advocated to prevent infection, but the exact effects it exerts on immune response and disease outcome is much debated. This review aims to provide an analysis from data of the mechanism of action of this supplement, the known immune response against COVID-19, as well as inflammatory and immune pathways already elucidated from similar infections such as SARS and MERS, in hope to gain a better understanding of the host immune responses and the possible outcome of its modulation. This predictive view may help in designing preventive measures against COVID-19 that can be widely adopted by the general population in the near future.

KEYWORDS COVID-19, immune response, cytokines, *echinacea purpurea*

Introduction

The rapidly-spreading pandemic of the novel virus SARS-CoV2 has infected more than 2 million people around the world, directly causing more than 150.000 deaths. [1] While the scientific community is on the record-speed race to invent vaccines and novel therapies, any definitive therapy or cure is still out of reach. Infection with SARS-CoV-2 has a relatively low case fatality rate (1.38%) compared to prior coronavirus infections such as SARS and MERS; however, it is particularly deadly towards susceptible populations with substantially higher ratios in older age groups (0.32% case fatality rate in those aged less than 60 years old compared to 6.4% in those aged 60 years old or older) [2]

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According to the latest medical knowledge, the participation of the immune system in the development and progression of COVID-19 is one of the key elements in the pathogenesis of the disease. [3] A robust immunity is essential in initial resistance against the disease; the elderly and the immunocompromised seem to be the most susceptible. It has been suggested that a successful innate immunity against the disease involves interferon (IFN 1) type responses and its downstream cascade. [4] However, SARS-CoV-2 seem to be able to exert a dampening effect on this initial response, allowing it to replicate faster than the immune system can handle- possibly explaining its long incubation period. At the same time, in severe or lethal cases, increased neutrophil and monocyte-macrophage is observed; in fact, dysregulated type I IFN and myeloid cells infiltrate seem to be the leading cause of lethal pneumonia. It is thought the immune response towards SARS-CoV-2 is divided into two phases: the initial adaptive response, and in severe cases, innate inflammation in the lungs that is primarily mediated by pro-inflammatory macrophages and granulocytes. [4] Therefore, any approach involving immune response should consider these two different phases.

Consumption of vitamins and "immune supplements" is

commonly advised to communities around the world in an added effort to curb this rapid outbreak. The purple coneflower *Echinacea purpurae*, a North American traditional medicine, has become a very popular supplement globally and some studies have shown it to possess antiviral and immunomodulating properties [5] *Echinacea* extract is shown to increase interferon-gamma production and increased percentages of CD 49+ and CD19+ lymphocytes in the spleen and NK-cell cytotoxicity. [6] On the other hand, *Echinacea* extract was also shown to mitigate the production of pro-inflammatory cytokines IL-1, IL-6, IL-8, and TNFα elicited by rhinovirus infection. [7] Secretion of pro-inflammatory cytokines by an assortment of viruses including HSV-1, influenza A virus, adenovirus type 3 and 11, and respiratory syncytial virus, was similarly attenuated by EP extract. [8] The role of EP in attenuating coronavirus-based respiratory infections, however, remains unknown. Due to the double-edged sword, the immune response plays in this disease, involving both robust initial immunity and later disease mechanism involving "cytokine storm", medications or supplements boosting immune response can at first glance be controversial. However, it is possible to dissect the issue further and provide accurate and current knowledge on the immune responses of COVID-19 infection, and especially the likely role of this common and readily accessible supplement in prevention or treatment of the disease.

Immune response in COVID-19 infection

The initial immune response against viral infection relies on innate immunity, especially the interferon type I system and its downstream cascade, which should then induce an adaptive immune response. RNA or other replication immediate, such as those in the case of the coronavirus, function as PAMPs (pathogen-associated molecular patterns) to be recognized as an invasion of the virus. This will lead to the activation of downstream signalling cascades such as NF- κB and IRF3, accompanied by their nuclear translocation, which will induce the expression of type I interferon and pro-inflammatory cytokines. [9] Via the JAK-STAT pathway, IFN will initiate the transcription of IFN stimulated genes, which should be able to suppress viral replication in the early stage successfully.

Both SARS-CoV and MERS-CoV seem to be able to suppress the type I IFN response, using multiple strategies involving both the type I IFN production and the downstream IFNAR signalling. SARS-CoV can interfere with the signalling through ubiquitination and degradation of RNA sensor adaptor molecules; via inhibition of IRF3 nuclear translocation; and inhibition of signalling even after IFN is secreted by decreasing STAT phosphorylation. [10] The viral proteins, including membrane (M) or nonstructural (NS) proteins (e.g. NS4a, NS4b, NS15), seem to be the key molecules in host immunomodulation. In the case of MERS-CoV, antigen presentation with both MHC class I and II was downregulated in infected immune cells, which would markedly diminish T-cell activation. It has been reported that SARS-CoV also directly infects macrophages and T cells, a key feature in SARS-CoV-mediated pathogenesis, although only minimal amounts of monocytes and macrophages in the lung express the ACE2 receptor. [10] Whether this virus can utilize other receptors for viral entry is still unknown. The genome of SARS-CoV2 also contains additional gene regions compared to SARS-CoV and MERS-CoV, and careful sequence comparison of these gene regions might uncover novel mechanisms. [11]

These dampening strategies seem to be closely associated with the prolonged incubation period and the disease severity. [12] SARS-CoV2 has a longer incubation period (average 2-11 days) compared to other viruses such as influenza (average 1-4 days). This long incubation period also complicates mitigation of this disease from an epidemiology standpoint, since patients who are still incubating and showing no symptoms might already be actively shedding and infectious.

SARS-CoV-2 seem to use the entry receptor of ACE2, [13], which is expressed on type 2 alveolar cells in the lung. The early pulmonary pathology of lung lesions seems to involve lung oedema, proteinaceous exudate, focal reactive hyperplasia of pneumocyte type II and the presence of multinucleated giant cells. These changes seem to happen even in patients who have yet to exhibit any symptom- this strongly corresponds to the long incubation period; which itself might further emphasize the dampening ability of this virus.

On the other hand, increased neutrophil and monocyte/macrophage influx is observed in severe cases of SARS-CoV and MERS-CoV. [10] SARS-CoV seems to show increased yet dysregulated type I IFN and inflammatory monocyte/macrophage, causing lethal pneumonia. This brings forth the theory that there are two phases of the immune response in severe SARS-CoV2 cases; an inadequate early innate immune response which allows for compromised viral control, and then an overactivated inflammatory response in the later stage, causing the so-called "cytokine storm".

In young children, where the early innate immunity is highly effective, almost no severe cases have been reported. [14] On the other hand, patients with underlying diseases compromising their innate immunity such as diabetes, hypertension, and cardiovascular disease, are highly susceptible to the disease. [15] This compromised innate immunity, causing delayed type I IFN response and unchecked viral replication in the early stage, might induce later hyperproduction of type I IFN and an influx of inflammatory innate immune cells, which are significant sources of pro-inflammatory cytokines.

Various studies indicated that lymphocytopenia (35-63%), increased total neutrophil (38%), and leukocytopenia (25%) is a characteristic finding in a large number of patients [13,15]. Increased serum IL-6 (52%) and increased C-reactive protein were also observed. [13] Increased neutrophil-to-lymphocyte ratio (NLR) seems to correlate with disease severity and death. [15,16]

In severe cases, a "cytokine storm" similar to SARS-CoV and MERS-CoV infection seems to be the underlying pathogenesis, manifesting as lung immunopathology, lethal pneumonia, and eventually acute respiratory distress syndrome. [15,17] An early rise in serum levels of inflammatory cytokines (e.g., IL1B, IL6, IL12, IFNγ, IP10, and MCP1) were observed in patients who end up with extensive lung damage and needing ICU care, suggesting the involvement of inflammatory cytokines in disease severity. This is similar to MERS-CoV infection, where an increased concentration of proinflammatory cytokines (IFNα, TNFα, IL15, and IL17) is also reported. The high amounts of IL1B, IFNγ, IP10, and MCP1 in SARS-CoV2 indicates activated T-helper-1 (Th1) cell responses; besides, patients requiring ICU admission had higher concentrations of GCSF, IP10, MCP1, MIP1A, and TNFα, suggesting that the cytokine storm was directly associated with disease severity. However, 2019-nCoV infection also initiated increased secretion of T-helper-2 (Th2) cytokines (e.g., IL4 and IL10) that suppress inflammation, which differs from SARS and MERS-CoV infection. [15]

These facts strongly support innate immunity as an essential factor of disease outcome, and interventions that may strengthen innate immunity might be valuable. Still, the timing of administration may be the key to make sure the response yielded is a protective one. [12]

The immunomodulatory effects of *Echinacea purpurea*

Echinacea is a genus consisting of nine species, with three: *Echinacea angustifolia*, *Echinacea pallida*, and *Echinacea purpurea* having been used as a traditional North American medicine. In keeping with the increased popularity of herbal medicines throughout the world; *Echinacea* has been one of the most popular, with a wide range of pharmacological activities researched in several studies. It has been shown to function as supportive therapy for upper respiratory infections, including the common cold, and possess palliative effects on inflammation, wound and tumour growth. [18]

However, although *Echinacea* is commonly understood by the common population as an "immune-boosting" supplement, the dual effect of the immune system in both defending against SARS-CoV2 infection, and mediating more severe outcomes, makes the consumption of these supplements controversial. *Echinacea* must induce immunity without overactivation innate immune cells and eliciting overproduction of proinflammatory cytokines.

Several studies have shown that *Echinacea* does induce activation of innate immune cells. There are several modes through which the extracts induce these effects: by increasing immune cell amount, migration of granulocytes, phagocytosis ability of macrophage and NK cell cytotoxicity, as well as increasing cytokine production. [19-24] *Echinacea* seems to transform cellular gene expression in human immune cells, exemplified by changes in NK cells and cell surface antigens in human blood cells treated with EP. [25] Several other studies have described changes in mRNA and protein levels of cytokine genes in human blood samples, dendritic cells, and cultured human monocytes treated with EP. However, one study found that the effects were influenced by the nature of the preparation. [26-28]

Echinacea also shows the function in modulating adaptive immunity, with a study demonstrating an increase in IgM response in mice. However, although few studies have analysed its effects on T cells, with a few studies finding an increase in CD4+ lymphocytes and stimulation of interferon production. [29,30]

A study by Zhai et al. showed that administration of *Echinacea* stimulates T-cell proliferation and NK cell cytotoxicity, as well as cytokine production. Interestingly, production of specifically anti-inflammatory cytokines seems to be increased; in particular for IL-4 and IL-10. [6] Alkylamides isolated from *Echinacea* also suppressed secretion of inflammatory prostaglandin and TNF-α from influenza-infected macrophage-like cells. However, the activity depends significantly on source material and treatment- ethanolic extract seems to mask this anti-inflammatory activity. [31] Another pathway through which *Echinacea* might inhibit "cytokine storm" is through suppression of NFκB and possibly TLR-4, therefore inhibiting inflammatory cytokines. [32]

Echinacea purpurea was able to reverse the production of proinflammatory IL-1, IL-6, IL-8, and TNFα induced by rhinoviruses. [7] This ability was again replicated for several other viruses including HSV-1, influenza A virus, adenoviruses and RSV, although not beta coronaviruses. [8]

It is important to note that in cell cultures, incubation with EP resulted in increased phagocytosis and secretion of cytokines- but interestingly, this happens only for peritoneal, alveolar, and spleen immune cells preparations, and the opposite is true for airway epithelial cells and fibroblasts where *Echinacea* instead exerts an anti-inflammatory effect. [22]

Another study found that while *Echinacea* stimulates the proliferation and function of uninfected macrophages; it instead exerts anti-cytokine effect towards macrophage cell lines that are already stimulated with bacterial lipopolysaccharide. The same effect was observed in the same cells stimulated with rhinoviruses. This further supports the theory that *Echinacea* preparations "restore" normal immune functions instead of merely boosting its capabilities. [7] This variable effect reemphasizes that the general perception of *Echinacea* being a simple immune "booster" or "stimulant" is incorrect, and the term "immune modulator" is a more apt description.

However, many of these studies are performed in cell culture preparations. In reality, different preparation methods and origins of the preparation and the nature of oral consumption may render different effects in vivo. A study by Rininger et al. [33] reported that preparations of *Echinacea* showed a more significant effect on cytokine production once exposed to a simulation of gastric and intestinal digestion protocol- which is relevant to normal consumption of *Echinacea*. However, the mechanisms remain unexplained. A simulation is done on in-vitro reconstituted three-dimensional nasal epithelium also shows that overlaying cells with a thin layer of *Echinacea* preparation successfully prevented or reduced infection with HcoV strains in the respiratory epithelium in a dose-dependent manner. [34]

Clinical randomized, double-blind, placebo-controlled trials on the common cold showed that volunteers receiving *Echinacea* preparations had shorter cold durations and fewer episodes- the same study found that infections by other respiratory viruses, including beta coronavirus strains, was reduced during prophylactic treatment with *Echinacea*. A study using a different delivery method, such as aerosol, has yet to be performed. [35]

An interesting finding to note is that herbal preparations of *Echinacea* seem to exert antiviral activities independent of its immune-modulating functions, which applied to both influenza, RSV and parainfluenza viral families, to the highly relevant pathogenic coronaviruses, SARS-CoV and MERS-CoV. This antiviral activity seems to happen in the extracellular phase prior to viral entry and during viral release- it was also found to show comparable results across strains. However, the exact mechanisms also remain unclear- the hypothesis is that *Echinacea* preparations alter viral components, presumably the membrane and membrane protein, since it seems to be especially effective in enveloped viruses. [36]

Conclusion

Several conclusions can be derived from these results. Probably the most important is that in efforts to prevent or treat COVID-19, we should take note of the two distinct phases of the immune response: the initial innate immune response, ideally as robust as possible to prevent disease progression; and later, the overactivation of immune response resulting in cytokine storm and possibly precipitating lung damage in severe cases, which should be avoided. In the case of *Echinacea purpurea*, while it is often known as an "immune-boosting" supplement, reviews on various studies show that the term "immune normalizing" is more apt since it both increases innate immune response while

also producing anti-inflammatory cytokines, which could provide benefit on preventing damage caused by a storm of proinflammatory cytokines. It is, however, essential to take note that there is a lack of clear information or regulation on the origins of commercially available *Echinacea* extract, as well as the method of extraction and preparation, which are essential as studies have shown that extracts made with different methods from different parts of the plant exert differing effects. Still, preparations of *Echinacea purpurea* extract shows promise as a possible adjuvant therapy or supplement in protecting against SARS-CoV2. Further in-vivo and larger studies will be needed to determine recommended doses and analyze its exact effects on preventing infection; however, theoretically, we could be reasonably optimistic that supplementation with *Echinacea* preparations can be another avenue in improving public resilience and health in the time of the pandemic.

Ethics committee approval

No human or animal experiments were conducted in this study. No participation consent was needed. All sources used for documentation were adequately mentioned concerning copyright laws.

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Conflict of interest

Author declare no conflict of interests.

Data availability

The authors declare that data supporting the findings of this study are available within the article.

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