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Opinion/Hypothesis | Therapeutics and Prevention

Could Unconventional Immunomodulatory Agents Help Alleviate COVID-19 Symptoms and Severity?

Stephen W. Mamber[1], Steven Krakowka[2], Jeffrey Osborn[3], Lloyd Saberski[4], Ryan G. Rhodes[4], Albert E. Dahlberg[6], Kara Fitzgerald[7], Neal Wright[8], Sarah Beseme[9] and John McMichael[1,9*]

1) **The Institute for Therapeutic Discovery, Delanson, NY**
2) **The Ohio State University, Columbus, OH**
3) **The University of Kentucky, Lexington, KY**
4) **Yale University, New Haven, CT**
5) **The University of North Carolina – Wilmington, Wilmington, NC**
6) **Brown University, Providence, RI (emeritus)**
7) **Sandy Hill Clinic, Sandy Hill, CT**
8) **CMC Biosciences, Beverly, MA**
9) **Beech Tree Labs, Providence, RI**

***Corresponding Author (jm@beechtreelabs.com). PLEASE NOTE - STEPHEN MAMBER IS CORRESPONDING AUTHOR THROUGH THE SUBMISSION PROCESS (rowr@comcast.net)**

ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS coronavirus 2 or SARS-CoV2) is the cause of the respiratory infection known as COVID-19. From an immunopathological standpoint, coronaviruses such as SARS-CoV2 induce an increase in a variety of T-helper 1 (Th1) and inflammatory cytokines and chemokines including

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interleukins IL-1, IL-6, CCL2 protein and CXCL10 protein. In the absence of proven antiviral agents or an effective vaccine, substances with immunomodulatory activity may be able to inhibit inflammatory and Th1 cytokines and/or yield an anti-inflammatory and/or Th2 immune response to counteract COVID-19 symptoms and severity. This report briefly describes four unconventional but commercially accessible immunomodulatory agents that could be employed in clinical trials to evaluate their effectiveness at alleviating disease symptoms and severity: Low-dose oral interferon-alpha, microdose DNA, low-dose thimerosal and phytocannabinoids.

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OPINION/HYPOTHESIS

Severe acute respiratory syndrome coronavirus 2 (SARS coronavirus 2 or SARS-CoV2) is a recently discovered coronavirus capable of causing the 2019-2020 respiratory infection known as COVID-19. Symptoms range from fever and coughing to pneumonia or severe respiratory distress. It is related to the coronaviruses responsible for severe acute respiratory syndrome (SARS) from 2002-2003 and Middle East respiratory syndrome (MERS), first reported in 2012. Worldwide, over 8,400 people became sick with SARS, of which over 800 died. For MERS, close to 2500 cases have been detected, with about 850 related deaths (data from World Health Organization and National Institutes of Health websites). COVID-19 is widespread and, in the midst of this global pandemic, as of late March 2020, there were over 600,000 confirmed infections and almost 28,000 deaths worldwide (for updates, see the Johns Hopkins University coronavirus COVID-19 dashboard: https://www.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6). To date, there are no proven agents capable of countering the virus, and vaccine candidates are in early clinical testing, with availability to the general public at least a year away.

From an immunological standpoint, coronaviruses cause increases in T-helper 1 (Th1) cytokine interferon (IFN)-gamma, inflammatory cytokines such as interleukins IL-1, IL-6 and IL-12, and related cytokines and chemokines including IL-8, chemokine (C-C motif) ligand 2 (CCL2 protein, also known as monocyte chemoattractant protein-1 or MCP-1) and C-X-C motif chemokine 10 (CXCL10 protein, also known as Interferon gamma-induced protein 10 or IP-10) (1–7). The “cytokine storm” mediated by these inflammatory and Th1 cytokines activate monocytes/macrophages and neutrophils and

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are responsible for the immunopathological consequences of the infection. It is recognized that hyper-inflammatory immune responses can result in increased disease severity and mortality. Therefore, inhibition of the hyper-inflammatory response is a definitive drug therapy objective.

It has been proposed that certain biological response modifiers, notably, cytokines IL-37 and IL-38, have the potential to inhibit pro-inflammatory cytokines such as IL-6 and/or induce an anti-inflammatory immune response or immunomodulatory response that could counteract COVID-19 patients' hyper-inflammatory responses (8–10). However, the time it might take to develop such cytokine products for the treatment of COVID-19 patients is unknown at this time. There is an urgent need for substances that can potentially counter the effects of SARS-CoV2 and alleviate the symptoms and severity of COVID-19. In the current situation, every avenue of health care that might be available to decrease morbidity, disease symptoms and severity and promote survival may be worthy of investigation. Accordingly, it is suggested that clinical trials could be conducted with certain substances with immunomodulatory activity from the realm of complementary and alternative medicine. These immunomodulatory agents, while unconventional in nature, offer potential treatment advantages that could augment or possibly be used in place of standard clinical treatments. Furthermore, these potential immunomodulatory agents may be readily available for utilization in clinical trials sanctioned by the US Food and Drug Administration (FDA) or other government drug regulatory agencies. This report discusses four such agents, which were selected based on prior research conducted by the authors, both independently and collaboratively.

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They are 1) low-dose oral interferon-alpha (IFN-alpha); 2) microdose DNA; 3) low-dose thimerosal; and 4) oral or inhalable (by inhaler, not by combustion) phytocannabinoids.

Low-dose Oral IFN-alpha

IFN-alpha is a cytokine that is a known inducer of antiviral immune responses. There have been commercially available, injectable versions of IFN-alpha (e.g., IFN-alfa-2b, or *Roferon*), approved by the FDA only for chronic hepatitis C and certain forms of cancer. *Roferon* is dosed at 3-9 million international units (IU) and has substantial side effects (see https://www.drugs.com/pro/roferon-a.html). In contrast, oral (oromucosal) administration of human or bovine IFN-alpha at low doses of 50 - 200 units has been investigated as a potential antiviral agent for several decades. There have been substantial *in vitro, in vivo* and human and veterinary clinical research studies involving the use of low-dose oral IFN-alpha against infections caused by herpes and influenza viruses, foot and mouth disease virus, and a variety of bovine respiratory viruses (11–13). From a mechanistic standpoint, low concentrations of IFN-alpha can regulate the expression of a variety of cytokine, chemokine and related genes involved in antiviral immune responses. In one such study involving peripheral blood mononuclear cells from calves treated with 50 or 200 units of oral IFN-alpha, the expression of 41 of 92 tested autoimmune and inflammatory response-associated genes were significantly up- or down-regulated (14). Using the Kyoto Encyclopedia of Genes and Genomes (KEGG) online database (https://www.genome.jp/kegg/), 12 of these genes were identified as involved in cytokine–cytokine receptor interactions. What was particularly intriguing was that seven of these genes (CSF1, CXCL12, FAS, IL2RA, IL6R, TNFRSF1A and

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TNFSF13B) were down-regulated at the 50-unit concentration, whereas five of these genes (IFNAR2, IL1A, IL1B, IL10, and IL10RB) were up-regulated at the 200-unit concentration. These data suggest that low-dose oral IFN-alpha can regulate the expression of specific immune response genes that may be relevant to the alleviation of COVID-19 symptoms. While a double-blind, FDA-authorized clinical trial of low-dose oral interferon as prophylaxis for influenza did not prevent acute respiratory illness in treated relative to control individuals, it did reduce symptom severity and was seen as beneficial to a subpopulation of patients (15). Currently, it is marketed as a nutraceutical under the trade name of *Paximune*^{R}. Given the body of existing research and the unmet medical needs of COVID-19 patients, and a favorable safety profile at these dose levels, it is believed that an FDA-authorized clinical trial of this substance specifically for reducing the symptoms and severity of respiratory symptoms in COVID-19 patients could be conducted in relatively short order.

Microdose DNA

Cystic fibrosis (CF) is a genetic disease characterized by abnormal, viscous mucus secretions. The viscosity of these secretions results from a high concentration of exogenous deoxyribonucleic acid (DNA) that is released from necrotic neutrophils (16). This observation resulted in the development of the DNA-degrading enzyme, DNase (Dornase alfa; *Pulmozyme*^{R}) as a treatment for CF symptoms (17). Since the presence of excessive neutrophils in the sputum of CF patients suggested an aberrant compensatory immune response, it was hypothesized that exogenous, sublingually administered DNA could be applicable as a neutralization therapy. This hypothesis was the basis for the development of a proprietary formulation of a low concentration of DNA

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fragments derived from salmon sperm DNA, otherwise referred to as microdose DNA (18, 19). The term ‘microdose’ was applied based on its oral (sublingual) administration in microgram-range doses (0.6 ug per dose, based on a 12 ug/ml DNA concentration and a drop volume of 50 ul). It was hoped that sublingual dosing with microdose DNA could decrease neutrophil necrosis and DNA release into the lungs, thus decreasing sputum viscosity. Microdose DNA was first utilized in evidence-based clinical testing of CF patients. This sublingual therapeutic approach subsequently was extended to patients with other respiratory diseases and otitis media. The specific mechanism(s) of action of microdose DNA have not been elucidated; at least five different hypotheses have been postulated. Among these hypotheses are the generation of beneficial immune responses through increases in anti-inflammatory cytokines or immunomodulatory changes in T-helper 1/T-helper 2 (Th1/Th2) cytokine ratios (19). There is some experimental evidence from studies of dogs with kennel cough that microdose DNA increases levels of the anti-inflammatory cytokine, IL-4 (S. W. Mamber, unpublished data). It has also been observed that the DNA fragments could contain oligodeoxynucleotides with the CpG motif (CpG ODNs), which are known to stimulate an immune response to viral infections (20). Under the clinical trial names HP-3 and ML-03, microdose DNA was tested in three separate FDA-approved, placebo-controlled, double-blind Phase II clinical trials, one for the treatment of CF, one for chronic bronchitis and one for chronic obstructive pulmonary disease (COPD). There were only 17 treatment patients and 20 patients on placebo in the CF clinical trial. Although underpowered to achieve statistical significance, a trend toward improvement was observed for three respiratory parameters. In the chronic bronchitis trial, 25 patients

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were administered microdose DNA, with 24 patients on placebo. Among other endpoints, there was a statistically significant improvement ($p = 0.007$) in forced expiratory volume ($FEF_{25-75\%}$), a measure of small airway function. Finally, in the COPD clinical trial, there were 23 patients randomized to microdose DNA, vs. 25 on placebo. There was a statistically significant outcome ($p = 0.019$) in a key endpoint, the six-minute walk test (19). All three clinical trials demonstrated the potential of microdose DNA in improving respiratory function in patients with different lung diseases. Moreover, there were no safety issues apparent in these trials. Though it was not developed further as a pharmaceutical agent for economic reasons, the current DNA-based therapeutic is being marketed as a nutraceutical and is being sold commercially as *Mucolyxir*^{TM}. The combined evidence-based and clinical trial experiences for various respiratory ailments, plus commercial availability, makes microdose DNA a viable candidate to test in clinical trials for treatment of COVID-19 respiratory symptoms.

Low-dose Thimerosal

Thimerosal (alternatively, Thiomersal) is an organomercury compound that is commonly used as a vaccine preservative. With a typical concentration of 0.01%, a 0.5 ml dose of vaccine contains 50 ug of thimerosal. Because of controversy surrounding the presence of thimerosal in vaccines and neurological diseases such as autism, the use of thimerosal in vaccines has been curtailed over the past 20 years (see https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/thimerosal-and-vaccines). However, some researchers have been intrigued by the possibility that very small doses of thimerosal (0.2 ug, or 1/250th of the amount present in a typical vaccine dose) can promote an antiviral immune response. In that regard, low-dose

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thimerosal might be considered to be a hormetic, a substance that is beneficial at low concentrations but inhibitory or toxic at higher concentrations. In terms of background, in 1979 J. B. Miller reported that influenza vaccine could also be used to treat herpes virus infections (21). In studying the components of the influenza vaccine, it was eventually determined that the anti-herpes activity was not related to any influenza virus component of the vaccine. Rather, it was the thimerosal that was responsible (22). Further research indicated that low-dose thimerosal was not acting directly against herpes, influenza or other viruses. Instead, low-dose thimerosal may be signaling an antiviral host response that is immunological in nature. While the mechanism of action is not clear, *in vitro* gene expression profiling experiments with human diploid fibroblast cells indicate that thimerosal at low concentrations (1.6-40 ng/ml) can regulate the expression of specific cytokine, chemokine and related immune response genes capable of mediating host immune responses to viral infections (S. W. Mamber, unpublished results). Thimerosal can inhibit herpes virus activity, based on *in vitro* experiments showing viral plaque reduction in treated human keratinocytes, but this is believed to result from innate cellular immune responses rather than direct antiviral effects (V. Gurel, unpublished results). Low-dose thimerosal is currently not commercially available. However, it has been employed in two FDA-approved, randomized, double blind placebo-controlled clinical trials to evaluate its safety and efficacy. The first trial, a Phase lla study, evaluated thimerosal for its ability to block progression to lesion in patients with recurrent oral herpes caused by dental trauma, while the follow-up Phase llb study evaluated the same indication in patients with herpes caused by exposure to ultra-violet radiation. While the individual clinical trials

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were under-powered and did not show statistically significant outcomes, the pooled outcome data from both studies that shared a common endpoint did achieve statistical significance (Beech Tree Labs, unpublished data). There has been little experience in employing low-dose thimerosal against coronaviruses to date. However, a favorable safety profile, plus the simple formulation and sublingual dosing of low-dose thimerosal, makes this an interesting candidate for a clinical trial to determine if it can effectively alleviate COVID-19 symptoms and severity. (Just to further ensure safety, in accordance with thimerosal-containing vaccine recommendations by the FDA, low-dose thimerosal should not be given to children under age six).

Phytocannabinoids

Phytocannabinoids derived from *Cannabis sativa*, such as cannabidiol (CBD) and 9-tetrahydrocannabinol (THC) have been shown to inhibit inflammatory and Th1 cytokines and/or promote an anti-inflammatory and Th2 immune response both *in vitro* and *in vivo* (23–25). As COVID-19 represents a respiratory disease with a dominant Th1 and inflammatory immune response profile, it has been postulated that cannabinoids represent a class of compounds with the potential to alleviate COVID-19 symptoms and severity by helping to decrease inflammation and restore a Th1/Th2 balance in the immune system. THC, for example, has been shown to shift the Th1/Th2 cytokine balance in human T cells to one favoring Th2 cytokines. Of particular interest was the inhibition of IFN-gamma production (23). CBD decreased inflammation in a mouse model of lung injury, with decreased production of pro-inflammatory cytokines and chemokines, including IL-6 (24). In preliminary studies, an oil extract from *Cannabis sativa* containing both CBD and THC up-regulated Th2 and anti-inflammatory genes

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such as IL4 (encoding IL-4) and PPARG (encoding peroxisome proliferator-activated receptor gamma) in human small airways epithelial cells *in vitro*. There were also certain genes involved in mucus overproduction or hypersecretion that were down-regulated. These included CLCA1 (encoding chloride channel accessory 1) and CMA1 (encoding mast cell chymase 1) (25). Preliminary *in vivo* testing in Caribbean Vervets (*Chlorocebus aethiops sabaeus*) indicated that the oil extract improved inspiratory lung functions (J. Osborn, University of Kentucky, manuscript in preparation). More research will be needed to determine which cannabinoid or cannabinoid mixture might be effective in treating COVID-19 symptoms, and at what concentrations. The method of drug delivery is also a consideration. Combustible products (i.e., smoking) is obviously contraindicated for patients with acute respiratory distress. Oral ingestion would be the logical delivery method. However, an oil-based product may be suitable as the active pharmaceutical ingredient (API) for direct inhalation therapy (e.g., utilization in handheld aerosol inhalers). API formulation incipient propellants often use natural oil components. Such a formulation would offer a convenient treatment method through nebulizer delivery to the lungs.

Comment

The four substances described here do not have, or are not expected to have, direct antiviral activity against SARS-CoV2 *in vivo*. (Phytocannabinoids may be an exception, pending further research, which actually would be a positive). Rather, they appear to be acting as immunomodulatory agents. Modulation of the immune response may be achieved through inhibition of inflammatory cytokines, production of anti-inflammatory cytokines, restoring Th1/Th2 balance or otherwise signaling cells to produce

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therapeutically beneficial cytokines, chemokines and related proteins. Accordingly, such treatments may have the potential to alleviate the immunopathological symptoms caused by SARS-CoV2. Based on existing *in vivo* and clinical experiences, the optimal use of these potential immunomodulatory agents would be at the first signs of disease symptoms, when there would be a better chance of reestablishing immune homeostasis. One further consideration is the potential disease-modifying utility these immunomodulators may have in patients with pre-existing health conditions, including chronic respiratory diseases such as chronic obstructive pulmonary disease (COPD). Such patients may be at the highest risk for severe morbidity and mortality from COVID-19. If formal clinical trials are not feasible, it is suggested that these substances be investigated in an observational manner under principles of informed consent and compassionate use.

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