Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: March 31, 2024

Citation: Ben CC, Chun-kwok W, et al., 2024. Innate Immunological Defense Offers Protection Before and After Vaccination, Medical Research Archives, [online] 12(3). https://doi.org/10.18103/mra.v12i3.5596

Copyright: © 2024 European Society of Medicine. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

DOI
https://doi.org/10.18103/mra.v12i3.5596

ISSN: 2375-1924

REVIEW ARTICLE

Innate Immunological Defense Offers Protection Before and After Vaccination

Chan Chung-lap Ben [1], Wong Chun-kwok [1,3], Leung Ping-chung *[1,2],

[1] Institute of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong
[2] State Key Laboratory of Research on Bioactivities and Clinical Applications of Medicinal Plants, The Chinese University of Hong Kong, Hong Kong
[3] Department of Chemical Pathology, The Chinese University of Hong Kong, Hong Kong

***Corresponding auth:** pingcleung@cuhk.edu.hk

ABSTRACT

Introduction: Vaccine protects the recipient against invading bio-organisms via the Adaptive Immunity System. Vaccination works well if the organism has a constant unchanging structure. However, if variants develop and keep changing, vaccination becomes ineffective. The COVID-19 pandemic has given us sufficient warnings that variant could be worrying. Additional measures to prevent infection are very much desired.

"Trained Immunity" has been described as a unique non-specific innate system of immunological defense outside the adaptive system. It could be stimulated after various types of infection or via the influence of immune-stimulating compounds like β glucan, which is a polysaccharide.

Use of Herbal Combinations in the Viral Epidemics: Reviewing China's multiple reports on the combined use of herbal treatment in the 2003 SARS epidemic and the current COVID-19, together with standard managements, we believe that herbs containing immuno-stimulants like β glucan could be producing immuno-boosting effects. Since 2020 we organized series of platform studies aiming to prove that the application of two medicinal herbs, viz. *Astragalus membranaceus and Coriolus versicolor (AM and CV)*, singly or combined with COVID-19 vaccination, could produce trained immunity effects.

First, we reviewed our experience gained in the 2003 SARS epidemic when we completed immunological platform studies on a herbal formula, followed by a clinical trial on hospital workers using the same herbal formula to test their infection rate compared with non-users. Results yielded very positive observations in both platform studies and clinical trial.

Subsequently, human peripheral blood mononuclear cells were used to react with the *Astragalus and Coriolus (AM & CV)* extracts to study whether the two herbs in the study formulation, which are rich in β glucan, were supporting the establishment of innate immunological defense. Then, special mice were orally fed with the extracts with or without vaccination to establish further *in-vivo* evidence. Comprehensive studies of blood changes and intestinal mucosal changes were recorded. Results of this study showed significant increases on the immune-related cytokine productions after simple consuming the herbal combinations. When vaccination was given together with the oral herbal supplements, stronger antibody activities were demonstrated.

Discussion: Our research efforts, starting from the 2003 SARS epidemic, to the present COVID-19 pandemic, illustrated that *in-vitro* and animal experiments, using extracts of *Astragalus and Coriolus (AM & CV)* as adjuvant compounds, the innate immunological activities could be boosted up. When vaccination was combined in the animal model, synergistic effects were obvious. The favorable results encourage further studies on the clinical applications on human volunteers.

Introduction

Vaccines have solved a lot of problems related to infections. Varicella vaccine has eliminated small pox. Children from birth to two years all receive vaccines for measles, tetanus, whooping cough and pertussis which have freed them from those infections ever since. The most remarkable example is the orally taken, polio-vaccine which eliminated polio from the earth! (1) All these successful stories convince us that vaccine is the real hope against infections. Nevertheless, there are the sad examples. HIV infection experts have tried for some forty years, no vaccine is yet available. Influenza vaccine which is so familiar to us, has changing efficacy ranging from 30-50% or lower. Tuberculosis has enjoyed generations of vaccine and yet after 70 years, has not reached the efficacy expected. More desperate examples include parasitic infections like malaria, schistosomiasis etc., which are "vaccine resistant". (2) Vaccine works well via the adaptive immunological system only when the invading organism remains stable. The COVID-19 pandemic has demonstrated disappointments when the virus underwent rapid variant conversions as was repeatedly reported by the WHO office. (3) The effective vaccine is meant for a specific target organism which would be destroyed via the mechanism of "Adaptive Immunity". (4) The vaccination should result in the promotion of favorable cellular and serological responses with specific sustained effects. The emergence of frequent variants challenges the effectiveness of the vaccine used. (5)

Our immunological system is responsible to combat invading organisms, and under normal circumstances, short of unusual virulent invasions, the system works well, without the need of any vaccine. It is this general immunological strength of infection resistance that keeps us away from being infected. The whole family could succumb to the COVID-19 infection when all the members have deficient immunological strength. On the other hand, it is not uncommon to find one or a few of the family members staying healthy in spite of other members contracting the infection. The healthy members must have more efficient immunological strength. This strong defense strength is innate with the healthy individual, not related to vaccination. The primary immunological response is taken care of by the Innate Cells which rapidly recognize the invading pathogens and start eliminating them through phagocytosis and initiating chain reactions in the process of activating the adaptive immuno system. It has been found that innate immuno responses could be enhanced and sustained following earlier exposure to infectious pathogens, resulting in immunological memory which helps to protect against re-infection. (6) Experimental evidences were collected in animal platforms of candida albicans infection. (7) Clinically, observations related to general resistance to infections after early life vaccinations against TB, measles, Polio and others have been made and studied, and sustained immunological defense was observed. Netea's research group in the Netherlands suggested the term "Trained Immunity" to signify this phenomenon. (8, 9) Can this innate immunological defense viz. "Trained Immunity" be stimulated and strengthened with simple therapeutic means? (10,11,12) Can "Trained Immunity" offer protection against infection beyond vaccination?

Our aim of research is to verify, through a review of our 2003 SARS experience, extending to the present need of further studies during the Pandemic, whether medicinal herbs are capable of supporting "Trained Immunity".

REVIEW OF PAST EXPERIENCE AND CURRENT ATTEMPTS DURING THE PANDEMIC

I. Studies Completed in the 2003 Viral Epidermic

Twenty years ago, there was the "Severe Acute Respiratory Distress Syndrome (SARS)" crisis in Hong Kong. While hospitals in China commonly used a large variety of herbal formulae together with modern drugs for the treatment of hospitalized patients, we believed that using herbal combinations for the protection of people at risk of disease contraction would be as important. Those are the hospital workers, relatives, and friends of the infected, who need preventive measures. During the climax of the SARS epidemic, hospital workers were under extreme stress because of the high risk of infection. We organized a clinical trial using an innovative herbal formula for two weeks with the aim of protecting them against the viral infection. Over 2,000 volunteers participated. Subsequent analysis showed 0 infection rate in the treated, compared with a 0.4% infection among those not taking the herbal formula. (13)

The zero-infection rate led us to believe that the herbal combination we used could have boosted up the innate immunity of the treated group. Subsequent experiments done in our laboratory using cellular and animal platforms did showed evidences of immuno-supportive effects. (14)

The pleasant experiences prompted us to follow the logic of "Trained Immunity" in an attempt of creating a preventive agent in the present COVID-19 pandemic.

The herbal formula we used could be a reasonable choice of investigation as a follow-up of the past experience. However, the complexity of the formula consisting of nearly twenty herbs was not favorable for intensive studies related to quality control and pharmacological investigations. We need to simplify the herbal combination to the least number, yet maintaining its effectiveness. Two herbs in the formula, namely *Astragalus membranaceus and Coriolus versicolor (AM and CV)*, have well reported evidences of immuno-supportive effects which prompted us to accept them as priorities.

Research studies have shown that certain compounds, like complex polysaccharides present in some fungi and medicinal plants, can augment immunological activities, particularly via the macrophage system. (15) The two herbs we selected, viz *Radix Astragalus and Coriolus versicolor*, had been found containing rich collections of β glucan which is well known to be supportive to immunological defense. Our prior engagement with the Sloan-Kettering Cancer Institute in New York, also supported us to select *AM and CV* as the major components. [16,17] On the other hand, some harmless human intestinal bacteria, e.g. Bacillus subtilis has been used in vaccine development, using as an adjuvant. The Bacillis bacterium produces spores also rich in β glucan. [18,19]

II. Recent Attempts to Create a Herbal Agent for the Prevention of Respiratory Infection

In our early study using a murine subcutaneous immunization model with Globo H and GD3 carbohydrates conjugated to keyhole limpet hemocyanin as an antigen, mixed with a panel of β-glucan–enriched natural products as immunological adjuvants, we found that *AM and CV* were the most active immune-stimulants with adjuvant activity.[13,14] We further found that the water-soluble β-glucans isolated from *AM* exhibited significant immunomodulating activities by stimulating the proliferation of human peripheral blood mononuclear cells and enhancing the innate immunity-related interleukin (IL)-1β, IL-12, IL-10, granulocyte macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor (TNF)-α production from monocytes in a dose-dependent manner.[20]

We believe that priming the innate immuno system with the *AM* and *CV* for nonspecific protection against infections together with the SARS-CoV-2–specific antibodies generated by vaccine may allow preactivation of preferable trajectory immuno responses that increases the protection against SARS-CoV-2 infection in the individuals, and mitigates the disease severity.

Our research on immunological studies were accomplished with standard *in-vitro* and *in-vivo* platforms in the Institute of Chinese Medicine at The Chinese University of Hong Kong. Exploration started with human derived peripheral immunological cells when they were tested against water extracts of the two herbs. Following this were mice experiments vaccinated with a COVID-19 vaccine, in an attempt of studying their serological states of immunological defense after vaccinations. Subsequent oral administrations of the herbal extracts should give further indications of their additional immuno-boosting effects.

(i) *In-vitro* Study: Induction of Innate Immunity Related Provocations in Human Monocytes

Human peripheral blood mononuclear cells were isolated from fresh buffy coasts obtained from healthy volunteers of the Hong Kong Red Cross Blood Transfusion Service. They were cultured in a suitable medium (RPMI 1640) with different combinations of *AM* and *CV* for 48 hours. Monocytes were further stimulated with SARS-COV-2 spike protein for 48 hours. The supernatant fluids were collected for multiplex bead-based immuno assays for cytokine studies. The levels of IL-1B, IL-12, IL10, TNFα were measured with Eliza kits. The cells were labelled with fluorochrome-conjugated anti bodies and analysed with flow cytometer.

When the immune-stimulating natural products *AM* and *CV* were incubated with spike protein in human monocytes, the production of TNF-α, IL-6, and IL-10 was significantly increased. For IL-1β, increased productions were observed in cells incubated with a combination of *AM* and *CV*, and productions of neutrophil chemokine CXCL8 were decreased. Proinflammatory CC chemokine CCL5 was also significantly upregulated. IL-12 was one of the predominant cytokines secreted from activated DCs, and a significant increase of inflammatory IL-12, IL-1β, IL-10, and TNF-α was observed. *AM* and *CV* promoted the immuno response with an immuno-adjuvant effect on DCs, which was the principal antigen presenting cells interacting with B cells for antibody productions. When *AM* or *CV* was combined and incubated with DCs, significant synergy on the cytokine productions was not observed.

(ii) Animal Study – Immunological responses to vaccination before and after Herbal consumption

Pathogen-free BALB/c mice were taken from the University Animal Services Centre and handled according to strict regulations.

BNT 162b2 mRNA vaccine was administered on the hind limb intramuscularly on day1, 15, 50 and 85. Effects of *AM and CV* were observed in a separate groups, to be compared with other groups combining with the vaccine, or vaccine alone.

When mice were fed with the water extracts of *AM and CV*, stronger antibody neutralizing activities against ancestral SARS-CoV-2 were observed when compared with mice vaccinated with BNT162b2 alone. Moreover, serum from both BNT162b2 vaccinated mice with or without *AM/CV* treatment at 1:100 could effectively inhibit the luciferase-mediated bioluminescence signal of the pseudo virus neutralization assay using HEK293/Human ACE2 stable cell line.

The antibody activities against the spike protein after the third and fourth BNT162b2 vaccination were similar, suggesting that an additional fourth vaccination has no additional beneficial effects in terms of antibody activities against spike protein. When mice were vaccinated with BNT162b2 4 times and fed with the water extracts of *AM and CV*, significantly stronger activities were observed in the serum diluted from 1:64,000 to 1:1,024,000 when compared with the mice vaccinated only with BNT162b2 ($P < 0.05$).

AM and CV could not significantly enhance the IgG binding activities of BNT162b2 vaccination against various Omicron subvariant spike proteins. [20]

Discussion

Documented reports in the field of specific immuno memory have demonstrated that B and T cell–mediated adaptive immunity following infections can be enhanced by a process called trained immunity. [21] Functional reprograming of monocytes has been described as the phenomenon of "Trained Immunity". [22] Using T and B cells depleted mice infected with C. albicans, it was found that they could be protected against reinfection in a monocyte-dependent manner. It is suggested that this protective effect is mediated by C. albicans through its fungal cell wall which in β-glucan which induced increases in the function of innate immuno cells with distinct features, notably higher pro-inflammatory cytokine responses to secondary unrelated pathogens. [23]

Monocytes can develop immunological memory, a widely recognized functional characteristic of innate immuno training, different from the memory expressed in adaptive immuno T and B cells. Upon a secondary immuno challenge, either homologous or heterologous, trained monocytes/macrophages exhibit a more robust production of proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α, than untrained monocytes. [24]

We have shown that *AM* and *CV* could stimulate monocytes and DCs to produce IL-1β, IL-6, and TNF-α and some other proinflammatory cytokines, which may facilitate the immuno responses of BNT162b2 in antibody production, thus enhancing the innate immunological defense against respiratory infections. In addition to β-glucan, astragal side IV and several flavonoids from AM have also been shown to possess significant adjuvant activities when combined with conjugate vaccines. [25]

In additional, we found the oral intake of *AM* and *CV* extracts could enhance the immuno responses of BNT162b2 vaccination in mice after the third vaccination against the ancestral COVID-19 and Delta variants spike proteins by 5.8- and 4.3-fold, respectively. Oral intake of *AM* and *CV* could improve the breadth of antigen binding capacity against SARS-CoV-2 ancestral and variant SARS-CoV-2 spike proteins. With the increase in antibody production in BNT162b2 vaccination against both ancestral and variant COVID-19 spike proteins, this additional enhancement activity of *AM* and *CV* may provide more robust immuno protective responses against the infection.

Since the COVID-19 pandemic, reports from China strongly recommended the use of complicated classical herbal formulae for hospital treatment. The overwhelming importance of hospital treatment has again diluted the importance of prevention. The role of prevention is totally given to the hopeful vaccines. This might be a suitable time, for the special aspects of immunological innate defense be seriously studied and be included into the immediate future planning, not only for COVID-19 prevention but also for other respiratory infections. Now that effective vaccinations have become routine recommendations, those at risk of infection before or after the vaccination could still benefit. [26,27,28,29,30]

Since the common aim is to boost up the defensive ability, with the subsidence of the pandemic research efforts could be focus a more on the general boosting of innate immunity.

Conclusion

We have selected two widely used, edible medicinal herbs *AM and CV* as the main components of a preventive agent against respiratory infection, basing on their historical record and current popularity, as well as their rich source of β glucan. Their ability to boost up the innate immunological defense of the body, enhanced much more so with oral Bacillus subtilis as an adjuvant, has given us much encouragement to further pursue on the development of the highly desirable innate immunological booster against respiratory infection. [25]

Our research efforts, starting from the 2003 epidemic to the current pandemic have offered us sufficient confidence, that using the immune-stimulating natural products *AM and CV* as an oral adjuvant, together with the mRNA vaccine BNT162b2 to improve the antigen binding activities against SARS-CoV-2 infection in mice, could achieve favorable results in building up "Trained Immunity". [10, 11] Further studies are being planned on the clinical applications for human volunteers.

Conflicts of Interest Statement

The authors declare that they have no conflict of interest in publishing this article.

Funding Statement

This work was supported by the State Key Laboratory of Research on Bioactivities and Clinical Applications of Medicinal Plants and The Innovation and Technology Fund Partnership Research Programme of the Innovation and Technology Commission, Hong Kong (Project No.: PRP/008/21FX)

Conflict of interest statement. The authors declare that they have no conflict of interest in publishing this article.

Acknowledgments

Nil

References

1. Ada GL. The immunological principles of vaccination. *Lancet*. 1990;335(8688):523-526. https://doi.org/10.1016/0140-6736(90)90748-t
2. Ledford H. How does COVID-19 kill? Uncertainty hampers doctors' ability to choose treatment. *Nature*. 2020;580:311-312.
3. WHO. COVID-19. *Weekly Epidemiological Update*. September 2023.
4. Li C, Lee A, Grigoryan L, et al. Mechanisms of innate and adaptive immunity to the Pfizer-BioNTech BNT162b2 vaccine. *Nat Immunol*. 2022;23(4):543–555. Doi:org/10.1038/s41590-022-01163-9
5. Carabelli AM, Peacock TP, Thorne LG, et al. SARS-CoV-2 variant biology: immune escape, transmission and fitness. *Nat Rev Microbiol*. 2023;21(3):162–177. Doi:org/10.1038/s41579-022-00841-7
6. Janeway CA. *Immunology Garland Publisher*. 2001;USA 579
7. Hole CR, Wager CML, Castro-Lopez N, et al. Induction of memory-like dendritic cell responses *in vivo*. *Nat Commun*. 2019;10(1):2955. https://doi.org/10.1038/s41467-019-10486-5
8. Netea MG, Dominguez-Andres J, Barreiro LB, et al. Defining trained immunity and its role in health and disease. *Nat Rev Immunol*. 2020;20(6):375–388. https://doi.org/10.1038/s41577-020-0285-6
9. Netea MG, Giamarellos-Bourboulis EJ, Dominguez-Andres J, et al. Trained immunity: a tool for reducing susceptibility to and the severity of SARS-CoV-2 infection. *Cell*. 2020;181(5):969–977. https://doi.org/10.1016/j.cell.2020.04.042
10. van der Meer JW, Joosten LA, Riksen N, Netea MG. Trained immunity: a smart way to enhance innate immune defence. *Mol Immunol*. 2015;68(1):40-4. Doi:10.1016/j.molimm.2015.06.019
11. Netea MG, Quintin J, van der Meer JW. Trained immunity: a memory for innate host defense. *Cell Host Microbe*. 2011;9(5):355-61. Doi:10.1016/j.chom.2011.04.006
12. Quintin J, Saeed S, Martens JHA, et al. Candida albicans infection affords protection against reinfection via functional reprogramming of monocytes. *Cell Host Microbe*. 2012;12(2):223-32. Doi:10.1016/j.chom.2012.06.006.
13. Lau TF, Leung PC, Wong ELY, et al. Using herbal medicine as a means of prevention experience during the SARS crisis. *Am J Chin Med*. 2005;33(3):345-56. Doi:10.1142/S0192415X05002965
14. Chan CLB, Wong CK, Leung PC. What can we do for the personal protection against the CoVID-19 infection? Immuno-boostering specific supplement could be the answer. *J Emerg Med Trauma Surg Care*. 2020;2(1): 007.
15. Fung KP, Leung PC, Tsui KWS, et al. Immunomodulatory activities of the herbal formula Kwan Du Bu Fei Dang in healthy subjects: a randomised, double-blind, placebo-controlled study. *Hong Kong Med J*. 2011;17(Suppl 2):S41-3 (RFCID project number: 02040332).
16. Ragupathi G, Yeung K Simon, Leung PC, Lee YH Mavis, Lau BSC. Evaluation of widely consumed botanicals as immunological adjuvants. *Vaccine*. 2008;26(37):4860-4865. Doi:10.1016/j.vaccine.2008.06.098.
17. Feng H, Xiao Weilie, Ragupathi G, et al. The known immunologically active components of *Astragalus* account for only a small proportion of the immunological adjuvant activity when combined with conjugate vaccines. *Planta Med*. 2011;77(8):817–824.
18. Netea MG, Quintin J, Vander Meer JW. Trained immunity: a memory for innate host defense. *Cell Host Microbe*. 2011; 9(5):355-61. Doi:10.1016/j.chom.2011.04.006.
19. Leung PC, Chan CLB, Wong CK. Clinical use of Chinese medicine in the current COVID-19 crisis and related research planning. *Clin Res Infect Dis*. 2020,5(1): 1054.
20. Chan BC, Li P, Tsang MS, Sung JC, Kwong KW, Zheng T, Hon SS, Lau CP, Ho RC, Chen F, Lau CB, Leung PC, Wong CK. Adjuvant activities of immunostimulating natural products: Astragalus membranaceus (Fisch.) Bge. and Coriolus versicolor in BNT162b2 vaccination against COVID-19 infection. *J Leukoc Biol*. 2024;115(1):177-189. Doi:10.1093/jleuko/qiad106.
21. Gyssens IC, Netea MG. Heterologous effects of vaccination and trained immunity. *Clin Microbiol Infect*. 2019;25(12):1457-1458. Doi:10.1016/j.cmi.2019.05.024.
22. Pellon A, Nasab SDS, Moyes DL. New insights in Candida albicans innate immunity at the mucosa: toxins, epithelium, metabolism, and beyond. *Front Cell Infect Microbiol*. 2020;10:81. https://doi.org/10.3389/fcimb.2020.00081
23. Branko C, L.CJ. de Bree, Laszlo G, et al. BCG vaccination in humans elicits trained immunity

via the hematopoietic progenitor compartment. *Cell Host Microbe*. 2020;28(2):322–334 e5. https://doi.org/10.1016/j.chom.2020.05.014

24. Pérez-Hernández CA, Kern CC, Butkeviciute E, et al. Mitochondrial signature in human monocytes and resistance to infection in C. elegans during fumarate-induced innate immune training. *Front Immunol*. 2020;11:1715. Doi:10.3389/fimmu.2020.01715
25. Geller A, Yan J. Could the induction of trained immunity by β-glucan serve as a defense against COVID-19? *Front Immunol*. 2020;11:1782. Doi:10.3389/fimmu.2020.01782
26. Leung PC, Chan CLB, Wong CK. What do we expect from traditional Chinese medicine in the COVID-19 pandemic? *Chinese Traditional Medicine Journal*. 2020;3(1):1-5. https://bit.ly/2S8PmbW
27. Leung PC. A response to Massimo Bonucci's editorial: COVID-19 outbreak: how to use current knowledge to better treat. *EJMO*. 2020;4:2253-255. https://bit.ly/2FZ97Af
28. Leung PC, Chan CLB, Wong CK. Clinical use of Chinese medicine in the current COVID-19 crisis and related research planning. *Clin Res Infect Dis*. 2020;5(1):1054.
29. Chan CLB, Wong CK, Leung PC. The pandemic is calling for effective personal protection through innate immunological boosting. *J Vaccines Immunol*. 2021;7(1):009-013. Doi:10.17352/jvi.000039
30. Leung PC, Wong CK, Chan CLB. Therapeutic protection against COVID-19 infection while waiting for herd immunity. *Journal of Community and Preventive Medicine*. 2021;4(1):1-6.