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INNATE IMMUNE MODULATION AND RESISTANCE TO COVID-19

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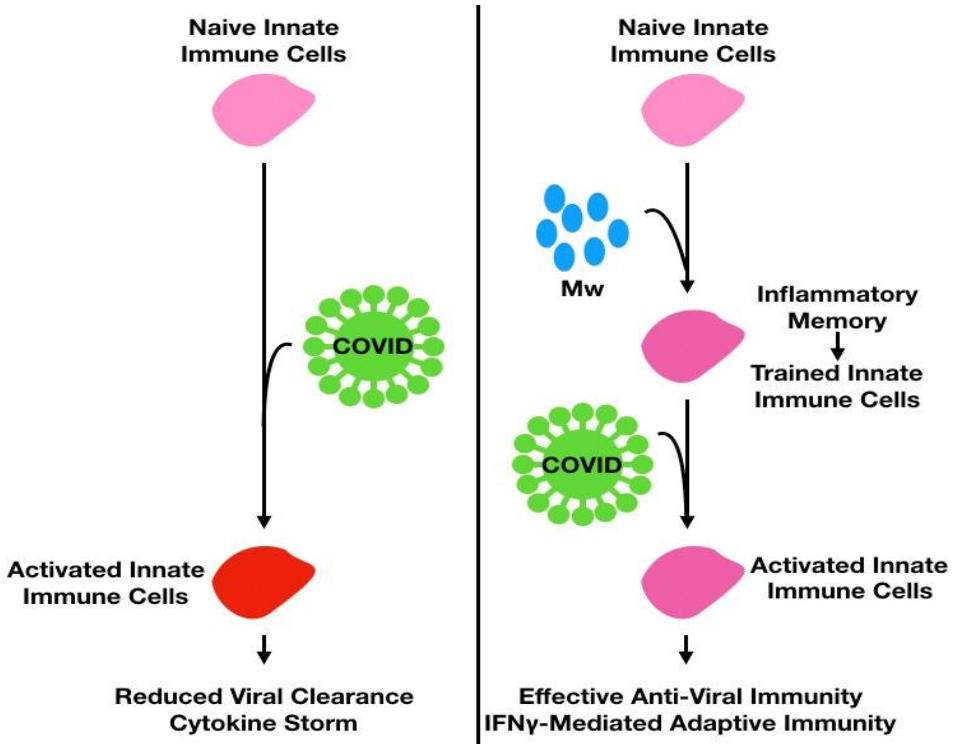
INTRODUCTION

Innate immunity is a fast, first-line defense against invading pathogens. Innate immune responses are responsible for initial defense against novel pathogens like COVID-19 and do not need the prior exposure required for adaptive immune responses. Innate immune cells such as macrophages and natural killer (NK)-cells detect pathogens entering body by recognising conserved pathogen-associated molecular patterns (PAMPs) and mount rapid responses to contain infection.[1] Th1 responses and IFN-γ secretion by macrophages and NK cells following PAMP detection are critical for anti-viral immunity.[2] IFN-γ is known to provide resistance to infection by coronavirus and its deficiency is associated with susceptibility to coronavirus.[3] IFN-γ also reduces the expression of Angiotensin Converting Enzyme-2 (ACE2) receptor, which is essential for the entry of coronavirus into the cell.[4]



In the management of COVID-19 transmission, boosting of the innate immune response via approved products such as Bacille-Calmette-Guerin (BCG) inoculation or other vaccines containing PAMPs has been suggested to provide resistance to COVID-19 infection and associated morbidity and mortality.[5,6] BCG inoculation is found to be associated with reduced upper and lower respiratory virus infections5. Like BCG, a heat-killed preparation of Mycobacterium w (Mw) is an approved immunomodulator in India. Mw is a potent TLR2 and NOD2 agonist with therapeutic activity in the setting of lung cancer, bladder cancer, and sepsis.[7-10] Live Mycobacterium w is a nonpathogenic organism. Treatment with Mw is associated with polarization of macrophages to an M1 phenotype with subsequent pure Th1-type adaptive immune responses.[11] Mw also

enhances NK cell mediated activity.[12] The efficacy of Mw is dependent on its ability to induce IFN-γ responses and is lost in IFN-γ knockout animals.[13] In a small controlled clinical study in a high-risk healthcare workers, Mw exposure was found to reduce the incidence of COVID-19.[14] In the current study, we evaluated the effect of Mw inoculation on the incidence of symptomatic COVID-19 in a large cohort.

RESULTS

This is a retrospective analysis of prospectively collected data for a cohort of 3831 subjects monitored for the development of symptomatic Covid-19 between June 1 and October 15, 2020. Of 3831 subjects studied, 2563 subjects (Mw group) volunteered to take Mw and 1268 (control group) were not inoculated. Of the 2563 subjects in the Mw group, 1663 received only one injection (0.1 ml intradermal in deltoid) while 900 received two injections (0.1 ml in deltoid over two weeks apart).

All subjects had their temperature measured once a day as per local government guidelines. Those who were found to have symptoms suggesting COVID-19 infection were tested for COVID-19 by RT-PCR as per guidelines. Those who had RT-PCR-confirmed COVID-19 infection were quarantined or hospitalised as per medical advice. All subjects are employees of the Cadila group of companies. Human resources departments of organisation coordinated Mw inoculation as well as COVID-19 diagnostic test results and subject hospitalization records.

Ethics: An independent external ethics committee approved this study.

RESULTS

Of 3831 subjects, 132 (3.45%) were diagnosed with COVID-19 confirmed by RT-PCR. The baseline characteristics of the 132 COVID-19+ subjects are shown in Table 1. The incidence of COVID-19 was 1.95% (50 of 2563) in the Mw group and 6.47% (82 of 1268) in the control group. All COVID-19+ subjects (132) had fever whereas dry cough, weakness, headache, loss of smell and taste, body ache, difficulty in breathing, sore throat and diarrhoea were more frequently seen in the Mw-free control group ($p<0.05$). Of the 132 COVID-19+ subjects, 57 were hospitalized and 75 were home quarantined. The incidence of hospitalisation was 0.51% (13 of 2563) in the Mw group and 3.47% (44 of 1268) in the control group.

Comparing the two groups, the hazard ratio of developing symptomatic COVID-19 and COVID-19 56 specific hospitalization was 0.297 (95% CI 0.209 -0.422; $p <0.0001$) and 0.144 (95% 0.078 to 0.268, $p<0.0001$) for Mw-treated groups, respectively. This translates to a protective efficacy (1 - HR) of 58 70.3% (95% CI 57.8 to 79.1) and 85.6% (95% CI 73.2 to 92.2), respectively. Of the 1663 subjects who received one injection of Mw, 29 (1.74%) developed symptomatic COVID-19. Of the 900 subjects who received two injections of Mw, 21 (2.33%) developed COVID- 19. The difference between these two sub-groups was not significant (odds ratio = 0.7474; 95% CI, 62 0.4237 to 1.3181; $p=0.3145$).

The incidence of symptomatic COVID-19 over time suggests that the prophylactic effect of Mw manifests quickly following the first administration of Mw (Figure 1) with widening of the gap between two arms over time, reflecting ongoing immune remodeling.

A local injection site reaction persisting for more than two weeks was seen in 690 (26.92%) subjects. This reaction was self-limiting and resolved without any specific treatment. No other side effects were seen.

Table 1: Baseline Characteristics.

symptoms	Mw Arm (%) N=50	Control Arm (%) N=82	p-value
Male / Female	49 / 1	76 / 6	-
Mean age (range)	38.5 (22 – 57)	38.33 (22 - 66)	-
Fever	50 (100%)	82 (100%)	0.244
Dry Cough	21 (42%)	59 (72%)	0.0008
Weakness	19 (38%)	52 (63%)	0.005
Headache	13 (26%)	50 (61%)	0.0002
Loss of smell and test	22 (44%)	71 (87%)	0.0001
Body ache	11 (22%)	65 (79%)	0.0001
Chest pain	11 (22%)	27 (33%)	0.1812
Difficulty in breathing	9 (18%)	29 (35%)	0.0356
Sore throat	7 (14%)	31 (38%)	0.0048
Diarrhoea	5 (10%)	22 (27%)	0.0293
Rashes on body surface	2 (4%)	11 (13%)	0.0969

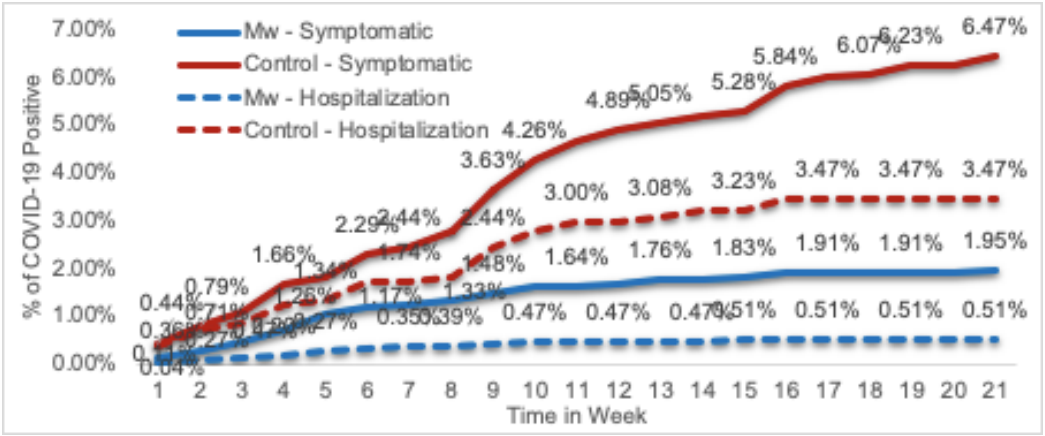


Figure 1: Incidence of symptomatic covid-19 and covid-19 specific hospitalisation over time. Top: Tracking incidence of symptomatic COVID-19 and covid-19 specific hospitalizationover time, we found that there were rapid and significant differences in the rates of symptomatic COVID-19 and covid-19 specific hospitalization between Mw-treated and control groups. Bottom: The trend towards Mw-mediated resistance to symptomatic COVID-19 was apparent and significant as early as week 1 and became more significant over the first 8 weeks of the study period.

Supplementary table: COVID-19 incidence and odds ratio per group. 126.

Week post immunisation	Cumulative incidence in mw group		Cumulative incidence in control group		Odd's ratio	95% CI	p-value	Relative Risk	95% CI	p-value
	%	No.	%	No.						
1	0.11%	3	0.44%	6	0.2474	0.0618 to 0.9907	P =0.0485	0.2474	0.0620 to 0.9875	P = 0.0479
2	0.27%	7	0.79%	10	0.3463	0.1315 to 0.9119	P =0.0318	0.3463	0.1321 to 0.9077	P = 0.0310
3	0.47%	12	1.10%	14	0.4241	0.1956 to 0.9195	P =0.0298	0.4241	0.1967 to 0.9141	P = 0.0286
4	0.70%	18	1.66%	21	0.4241	0.2251 to 0.7987	P =0.0079	0.4241	0.2268 to 0.7930	P = 0.0072
5	1.05%	27	1.81%	23	0.5808	0.3317 to 1.0170	P = 0.0573	0.5808	0.3344 to 1.0087	P = 0.0537
6	1.17%	30	2.29%	29	0.5118	0.3058 to 0.8564	P = 0.0108	0.5118	0.3086 to 0.8488	P = 0.0095
7	1.25%	32	2.44%	31	0.5107	0.3102 to 0.8407	P = 0.0082	0.5107	0.3131 to 0.8331	P = 0.0071
8	1.33%	34	2.76%	35	0.4806	0.2984 to 0.7742	P = 0.0026	0.4806	0.3012 to 0.7668	P = 0.0021
9	1.48%	38	3.63%	46	0.4087	0.2646 to 0.6314	P = 0.0001	0.4087	0.2674 to 0.6247	P < 0.0001
10	1.64%	42	4.26%	54	0.3848	0.2557 to 0.5791	P < 0.0001	0.3848	0.2586 to 0.5727	P < 0.0001

DISCUSSION

The reduction in incidence of symptomatic COVID-19 diagnoses and COVID-19 specific hospitalizations in the group receiving Mw inoculations seen in this study suggests that it is possible to increase resistance against development of COVID-19 by boosting the innate immune response. The protective efficacy seen in the Mw-treated group of 70.3% for COVID-19 diagnosis meets the WHO guidelines for protective efficacy of >50%. It is also identical to the efficacy seen with ChAdOx1 nCoV-19 vaccine.[15] The protective efficacy seen upon Mw exposure in this study may be due to the ability of Mw to trigger strong TLR2 signalling, polarisation of macrophages to a M1 phenotype, activation of NK cells, and increased IFNγ secretion and associated improved antiviral immune response. In accordance with previous studies using Mw, no systemic side effects were observed, suggesting that Mw may be a well-tolerated immunomodulator and amenable to broad use. Finally, that significant differences in COVID-19 diagnoses emerged between Mw- treated and -untreated groups in as little as 1 week post-inoculation is also in line with current understanding of innate immunity's fast-acting and long-lasting attributes, further

underscoring its clinical applicability. This is in contrast to time taken for the initiation antigen-specific adaptive immune response following administration of vaccine.

This is a limited retrospective observational study and findings should be considered indicative and not definitive. These results need to be replicated in a prospective randomized controlled trial before Mw can be recommended as an effective measure to provide resistance against COVID-19.

Conclusion: This study suggests that modulation of innate immune responses using Mw, an approved immunomodulator, is a promising strategy to reduce incidence of symptomatic COVID-19 infection and/or mitigate the development of COVID-19-associated morbidity until a vaccine can be administered.

REFERENCES

1. Chow, K. T., Gale Jr, M., & Loo, Y. M. RIG-I and other RNA sensors in antiviral immunity. Annual review of immunology, 2018; 36: 667-694.
2. Morris KV. The Road Less Traveled: SARS-CoV-2 and Cell-Mediated Immunity. Mol Ther, 2020 Dec 2; 28(12): 2521-2522.
3. Tau G, Rothman P. Biologic functions of the IFN-gamma receptors. Allergy, 1999 Dec; 54(12): 1233-51.
4. De Lang A, Osterhaus AD, Haagmans BL. Interferon-gamma and interleukin-4 downregulate expression of the SARS coronavirus receptor ACE2 in Vero E6 cells. Virology, 2006 Sep 30; 353(2): 474-81.
5. O'Neill, L. A., & Netea, M. G. (2020). BCG-induced trained immunity: can it offer protection against COVID-19?. Nature Reviews Immunology, 20(6): 335-337.
6. Chumakov K, Benn CS, Aaby P, Kottilil S, Gallo R. Can existing live vaccines prevent 141 COVID-19? Science, 2020 Jun 12; 368(6496): 1187-1188.
7. Pandey RK, Sodhi A, Biswas SK, Dahiya Y, Dhillon MK. Mycobacterium indicus pranii mediates macrophage activation through TLR2 and NOD2 in a MyD88 dependent 144 manner. Vaccine, 2012; 30(39): 5748-5754.
8. Belani CP, Chakraborty BC, Modi RI, Khamar BM. A randomized trial of TLR-2 agonist CADI-05 targeting desmocollin-3 for advanced non-small-cell lung cancer. Ann Oncol, 2017 Feb 1; 28(2): 298-304.
9. O'Donnell, M. A., Singh, S., Sood, R., Amlani, J., Krishnamoorthy, H., Shukla, K., & Modi, R. (2019). A clinical trial of the intradermal TLR2 agonist CADI-05 for BCG recurrent and unresponsive non-muscle invasive bladder cancer. Bladder Cancer, 151; 5(2): 71-180.
10. Sehgal IS, Agarwal R, Aggarwal AN, Jindal SK. A randomized trial of Mycobacterium w in severe sepsis. J Crit Care, 2015 Feb; 30(1): 85-9.
11. Banerjee S, Halder K, Ghosh S, Bose A, Majumdar S. The combination of a novel immunomodulator with a regulatory T cell suppressing antibody (DTA-1) regress advanced stage B16F10 solid tumor by repolarizing tumor associated macrophages in situ. Oncoimmunology, 2015 Jan 30; 4(3): e995559.
12. Ahmad F, Mani J, Kumar P, Haridas S, Upadhyay P, Bhaskar S. Activation of anti-tumor immune response and reduction of regulatory T cells with Mycobacterium indicus pranii (MIP) therapy in tumor bearing mice. PLoS One, 2011; 6(9): e25424.
13. Rakshit S, Ponnusamy M, Papanna S, Saha B, Ahmed A, Nandi D. Immunotherapeutic efficacy of Mycobacterium indicus pranii in eliciting anti-tumor T cell responses: critical roles of IFNγ. Int J Cancer, 2012 Feb 15; 130(4): 865-75.
14. Jaiswal, S. R., Mehta, A., Bhagwati, G., Lakhchaura, M. R., Aiyer, H., Khamar, B. M., & Chakrabarti, S. (2020). Innate Immune Response Modulation and Resistance to SARS- CoV-2 infection: A Prospective Comparative Cohort Study in High Risk Healthcare Workers. medRxiv.
15. MN, Minassian AM, Ewer KJ, Flaxman AL, Folegatti PM, Owens DR, Voysey M, Aley PK, Angus B, Babbage G, Belij-Rammerstorfer S, Berry L, Bibi S, Bittaye M, Cathie K, Chappell H, Charlton S, Cicconi P, Clutterbuck EA, Colin-Jones R, Dold C, Emary KRW, Fedosyuk S, Fuskova M, Gbesemete D, Green C, Hallis B, Hou MM, Jenkin D, Joe CCD, Kelly EJ, Kerridge S, Lawrie AM, Lelliott A, Lwin MN, Makinson R, Marchevsky NG, Mujadidi Y, Munro APS, Pacurar M, Plested E, Rand J, Rawlinson T, Rhead S, Robinson H, Ritchie AJ, Ross-Russell AL, Saich S, Singh N, Smith CC, Snape MD, Song R, Tarrant R, Themistocleous Y, Thomas KM, Villafana TL, Warren SC, Watson MEE, Douglas AD, Hill AVS, Lambe T, Gilbert SC, Faust SN, Pollard AJ; Oxford COVID Vaccine Trial Group Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. Lancet, 2020 Nov 18; 396(10267): 1979–93.