Tempering Macrophage plasticity for controlling SARS-CoV-2 infection for managing COVID-19 disease

Devinder Toor¹, Aklank Jain², Shivani Kalhan³, Harmesh Manocha⁴, Vivek Kumar Sharma⁵, Payal Jain⁶, Vishwas Tripathi⁷ and Hridayesh Prakash^{1*}

- 1. Amity Institute of Virology and Immunology, Amity University Uttar Pradesh, Sector 125, Noida, Uttar Pradesh, India**
- 2. Department of Zoology, Central University of Punjab, Bathinda, Punjab, India**
- 3. Department of Pathology, Government Institute of Medical Sciences, Greater NOIDA, UP, India**
- 4. Department of Microbiology Government Institute of Medical Sciences, Greater NOIDA, UP, India**
- 5. Department of Physiology, Government Institute of Medical Sciences, Greater Noida, UP, India**
- 6. Department of Medicine, Government Institute of Medical Sciences, Greater Noida, UP, India**
- 7. School of Biotechnology, Gautam Buddha University, Greater Noida, 201308, Gautam Budh Nagar, U.P.(India)**

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*** Address for correspondence**

Dr. Hridayesh Prakash

Amity Institute of Virology and Immunology,
Amity University Uttar Pradesh,
Sector-125, Noida, India
Phone: +91-120-4392961
Email: hprakash@amity.edu

Abstract

Hyper activation of macrophages contributes to acute respiratory distress syndrome, respiratory failure, and subsequent death of COVID-19 cases. Given this, tempering macrophage plasticity is paramount and the highest priority for the management of COVID-19 cases. In this context we here propose that either exchange or in situ re-programming of derailed Th17+ alveolar macrophages/ Slan+ DC with Th1 programmed counterpart would potentially mitigate or abolish pulmonary fibrosis. This approach is also anticipated to afford antiviral immune response and promote recovery in the patients and hold tremendous potential for managing severely infected patients by both curbing viruses and enhancing post-treatment recovery.

Background

The recent outbreak of the COVID-19 has challenged the health care system for developing effective and immediate therapeutics for controlling novel SARS-CoV-2 for the effective management of COVID-19 pandemic which is manifested by aberrant immunopathology. This is a new pathology and our knowledge about this disease is limited therefore lot of work is required before any therapeutics/vaccine could be launch for its eradication. Several recent and compelling studies have propose several aspects of pathological basis of disease (1) which enhanced our understanding how SARS-CoV-2 is interacting with alveolar and peripheral tissues and causing the death of the infected patients.

Role of macrophage in Pathogenesis of Covid 19

SARS-CoV-2 induces pathogenic inflammation, pulmonary fibrosis, acute respiratory distress syndrome (ARDS) and nephropathy which ultimately causing the death of infected patients within 2-3 weeks (1). Hyper-activation of committed macrophages which are termed as macrophage activation syndrome (MAS) (2) is associated with ARDS (3) in manifesting respiratory failure and subsequent death of COVID-19 patients. Autopsy reports and single-cell RNA sequencing (4) revealed the presence of monocyte-derived *FCN1*⁺ macrophages and other immune cells (4;5) in the broncho alveolar lavage fluid (BALF) of COVID-19 patients with severe ARDS. However due to abundance of macrophages over other immune cells and MAS in COVID 19 patients, these cells seems to be important for pathogenesis.

Transcriptional analysis of BALF further revealed high levels of IFN- γ induced protein-10 (IP-10) and various chemokines like MCP-1, CCL2, and CCL7 in BALF of severe COVID-19 patients which correlated with increased infiltration of monocyte / Macrophages (6) in their lungs. Cytokine profiling of critical COVID-19 patients reflects MAS with high levels of pro-inflammatory cytokines and chemokines such as IL-6, IL-7, Tumour Necrosis Factor (TNF- α), CCL2, CCL3, CXCL10, and IL-2 receptor. This demonstrate dysregulated activation of the mononuclear phagocyte system, hyper-inflammatory response (7) and MAS (8) which is causing death of patients. Based on

these compelling evidence tempering macrophage plasticity seems to be one of the most suitable interventions for effective management of COVID-19 disease.

Both activation and increased infiltration of monocytes, dendritic cells, neutrophils, and lymphocytes (9) during infection leads to ARDS and cytokine storm in COVID-19 patients. During SARS-CoV-2 infection, activated CD4⁺ T lymphocytes also secrete granulocyte-macrophage colony-stimulating factor which further stimulates macrophage to secrete pro-inflammatory cytokines thereby continuing the vicious cycle of the cytokine storm. SARS-CoV-2 activate alveolar, splenic and renal macrophages through angiotensin-converting enzyme 2 (ACE2) and enhance the secretion of IL-6, TNF- α , IL-10, and PD-1 (9) in patients. Both ARDS and MAS contribute to significant depletion of CD8⁺ CTLs which associated with disease severity (10) in COVID-19 patients. SARS-CoV-2 infects type 2 alveolar cells, epithelial cells and podocytes in lungs and kidney respectively and interacts with them via ACE2 receptors which facilitate the attachment and entry of this virus into host cells (11;12). ACE-2 / Angiotensin-II receptors are known to activate Sphingosine-1-phosphate receptor 1 (S-1P receptor 1) which is known to mediate IL-6 induced myopathy and fibrosis (13) S-1P R-1 signalling is associated with Th2 / 17 responses (14;15), hypoxia and allergic manifestations (16) which altogether promotes Th2 bias in the infected patients. Therefore it is rationale to presume that S-1P receptor 1 signalling would enhance the pathogenesis of COVID-19 cases. In view of this, blocking S-1P signalling either by application of S-1P lyase;(17) or FTY-720 / Fingolimod (18;19) seems rationale to modulate the pathogenesis of novel COVID-19 disease. In view of this FTY-720 is being explored in the Phase-2 clinical trial against COVID-19 patients (NCT04280588, MRCTA, ECFAH of FMU) and results are still awaited. However, due to its immunosuppressive nature, FTY-720 is expected to only lower down the hyper-inflammatory response and may afford only symptomatic relief in COVID-19 cases. Thus this may not afford clearance of SARS-CoV-2 infection (20) in COVID-19 cases.

Pre-clinical / Animal models for validation of proposed concept

Like any other new proposed interventions, validating new hypothesis in pre-clinical model is essential before its clinical application in the patients. Depending upon mouse

specific SARS-CoV-2 isolates, inbred mice like Balb/c & C57BL/6 represent suitable model of testing vaccines, antiviral drugs and disease pathogenesis (21). Furthermore due to dependency of SARS-CoV-2 on Ace-2(22), Tmprss2 (23) and Stat1 (24) protein for manifesting disease, knockout mice of these proteins also represent a potential model for validating concept against COVID-19 disease. Most interestingly, floxed strain of these mice could be used and backcrossed with LysM Cre mice to address macrophages related phenotype. To demonstrate anti-Covid 19 potential of M1 macrophages, most suitable strategy is to either use clinical isolate of SARS-CoV-2 or express major spike proteins of this virus in VeroE6 cell line and co culture them with THP / CD14+ / CD11b+ macrophages and evaluate the influence of various adjuvants mentioned in Table -1 / Figure 1 on innate and adaptive immune response of both macrophages and CD8+CTL. Several aspects like maturation, M1 / Th1 programming of macrophages and subsequent killing of Virus / expression of Spike proteins can be used to correlate the M1 programming of RAW / THP / CD14+ / CD11b+ primary macrophages. Likewise T cell response could be measured by co culturing T cells with infected epithelial / macrophages co culture. Various parameters like TARC/CCL17, RANTES STAT-1, 6, calcineurin, IRF - 4, 8 factors Th1 / Th17 response could be evaluated for addressing the impact of M1 programmed macrophage on T cell activation and killing of infected epithelial cells. These per-clinical experiments are essential for validating anti-Covid impact of M1 / CD8+CTL. These approaches certainly represent most suitable approaches for validating the concept at pre-clinical levels and need further investigations

Translational Approach

In view of above facts, mitigating pathogenic inflammation (Th2/17 responses) and pulmonary fibrosis is a paramount requirement for the management of COVID-19 infection. In this context our previous studies have potentially demonstrated the significance of iNOS+M1 effector macrophages and CD8+cTL in controlling respiratory pathogens (6-8), which are associated with community-acquired pneumonia. On the basis of these studies, we believe that tweaking these effector immune cells may be sufficient to control replication of SARS-CoV2 infection as well in COVID-19 cases. This

can be afforded either by their in situ reprogramming of Th2/ Th17 programmed macrophages or exchanging Th2/17 programmed macrophage with Th1 primed iNOS+CD14+M1 macrophages and CD8+cTL's in neo-adjuvant setting. Based on this and our previously published studies (9;10) we also believe that the exchange of Th17+ macrophages with Th1+ M1 macrophages may also afford protective immunity against SARS-CoV-2 infection in Covid 19 cases. This approach is anticipated to change the fate of severely infected patients but needs to be commissioned before its clinical application.

Proposed intervention

While the application of antiviral drugs like Remdesvir, Tocilizumab is sufficient to control the infection. Whether these drugs are adequate to augment immunogenic inflammation and clear infected cells from the infected organ is questionable. In this context, we have proposed the potential role of Ceramide-1 phosphate as immune adjuvants for promoting Th1 programming of infected cells/ tissue and subsequent clearance of viral infected cells from the tissue (currently under revision). In view of potential involvement of S1P in fibrosis and Th1 bias, FTY720 based interventions is currently under Phase-2nd clinical trial (NCT04280588) which is based on trapping effector T cell in the lymph node for effective control of infection and mitigating cytokine storm. However due to immunosuppressive nature, we believe that FTY-720 may not afford help to the patients. Likewise other studies have also highlighted the significance of BCG vaccination with increased resistant to the infection (25) and demonstrated reduced mortality in dexamethasone treated (26) COVID 19 patients. Since these interventions are targeted to reduce inflammatory score and anticipated to provide only symptomatic relief therefore other more robust and stable interventions are required for managing disease.

Moreover several reports mentioned above have provided compelling evidence that hyper activated macrophages are critical for ARDS and subsequent death in the patients so considering these aspects , combining macrophage directed strategies with other available approaches (as adjunct approach) is decisive for effective management of severely infected cases thus certainly deserve attention. Out of several available

options, most relevant strategy would be to promote in situ reprogramming of Th2/17 macrophages toward M1 by employing various neo adjuvants which are summarized in Table-1

In past we have identified several neo adjuvants like PDE4b/ AC mimetic which are capable of modulating Th17 response in human CD14+ monocyte and Slan DC (27), which complies with one of the recent study (3) which highlighted the potential of androgenic receptors in modulating cytokine storm. Therefore, we feel that PDE4/AC modulators (28) can also potentially modulate/mitigate macrophage activation syndrome and afford help in COVID-19 cases.

Apart from these, we have validated several other immune adjuvants like low dose Gamma Radiation (29;30), S-1P (31), and Smac mimetics (32) bearing potential of Th1 programming of macrophages in pre-clinical models system. Therefore we believe that these adjuvants are likely to afford immunity against SARS-CoV-2 infection as well as in available animal models which we have discussed above. Inclusion of various other adjuvants mentioned in **Table-1** targeting refractory macrophages, can also be potentially utilized for tempering macrophages plasticity for controlling SARS-CoV-2 infection effectively in COVID 19 cases.

Macrophages/ MSC as palliative regimes for COVID 19

Since macrophages are fairly plastic component of the body and could afford help for tissue regeneration and homeostasis after completion of therapy. These aspects are critical for the expected outcome of proposed immune / pharmacological interventions against infection. Apart from this, M2 or refractory macrophages also known to promote activity of fibroblast / mesenchymal stem cells/fibroblast (33;34) which can potentially neutralize therapy related inevitable adverse impacts. Both MSC and macrophages secrete various factors (35;36) which are involved in wound healing. On account of regenerative potential of macrophages (37;38) and their close association with mesenchymal stem cells (39;40) these cells can potentially be utilized as palliative approach (41;42) for accelerated recovery of patients. This certainly warrants the use of

macrophages / MSC as whole-cell infusions or their products as palliative components for enhancing recovery of COVID 19 patients post interventions.

Major Perspective

The proposed interventions which is depicted in **Figure 1** seem to be the candid approach for tuning pathogenic inflammation, Th1 reprogramming of derailed Macrophages, enhancing MHC-I dependent presentation of viral antigens to CD8+cTL for augmenting required adaptive immunity of host against SARS-CoV-2 infection. This is a novel immunotherapeutic approach and translationally viable in nature. The approach harbour potential for effective management of SARS-CoV-2 infection in severely infected COVID-19 patients. We also envisage that such an approach could be employed as potential adjunct to placid / antiviral therapies for effectively manage COVID 19 disease (both prospective and therapeutic in patients) thus certainly deserve immediate investigation for its clinical efficacy.

Conflict of Interest:

The author declare that they do not have any conflict of interest which need to be declared

Author Contributions

HP, conceived the idea and supervised the entire study.
DT, AKJ, SK, HM, VS, contributed to research tool
DT and VT: Critical analysis
HP and AJ wrote the manuscript.

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Figure 1: Schematic representation of augmenting SARS-Co-V2 specific adaptive immunity

Shown here are the most potent immune mediate (perspective) and/or pharmaceutical / palliative approaches for augmenting adaptive immune responses of derailed Macrophages / S1anDC / CD8+ T cells for the treatment and recovery of COVID-19 patients. These approaches are paramount requirement (as conjunction therapies) and expected to afford help for managing Covid 19 disease.

Table 1**Clinical trials targeting Activated macrophages for indicated inflammatory and tumor Diseases**

Clinical Trials	TAM Targeting agent	Target	Clinical Phase	Disease	Impact
NCT03123783	APX005M	CD40	I/II/Active	NSCLC	Macrophages activation
NCT01433172	(GM.CD40L) vaccine with CCL21	CD40	I/II/Active	Lung cancer	Targeting CD14+/CD16+ alveolar macrophages
NCT02637531	IPI-549	PI3K γ	I/ Active	Advanced solid tumor; non-small cell lung cancer; melanoma; breast cancer	Macrophage plasticity
NCT00397826	Simvastine	p38MAPK	II/Active	COPD	Macrophages activation
NCT04280588	FTY 720 (Fingolimod)	S1P	II/Active	COPD, Multiple sclerosis	proposed as a prophylactic agent against Covid19
NCT01103635	ICINCB7839	ADAM17	I/ Active	Liver Cirrhosis	Dampening Kuffer cells activation
NCT04261075	IPH5201 and IPH5301	CD39 & CD73	I	Cancer	Activation of macrophages and T cells

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