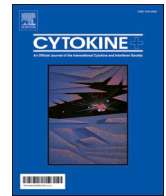




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COVID-19 imparted immune manifestation can be combated by NLGP: Lessons from cancer research

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ABSTRACT

SARS-CoV-2 easily infects human monocytes, macrophages and possibly dendritic cells (DCs), causing dysfunctions of these important antigen presenting cells (APCs). Observed DC dysfunctions facilitate improper antigen presentation, which obviously results T cell anergy, exhaustion and apoptosis, thus, may be contributing significantly in SARS-CoV-2 infection associated lymphopenia. Neem Leaf Glycoprotein or NLGP has enormous role in altered DC functions, thereby, offering optimum T cell mediated cytotoxicity, as experienced from cancer system. Such NLGP guided correction of altered DCs might also be effective to generate proper SARS-CoV-2-specific effector and central memory T cells.

In March 2020, World Health Organization (WHO) declared coronavirus disease 2019 (COVID-19) as a Pandemic because such disease affected so far 185 countries. Every global sector is looking for the remedy from this severe health and economy threatening disease. We are now aware of some immunological manifestations of SARS-CoV-2 within human bodies, which causing alarming respiratory threat. Very basic hemato-immunological symptom of this disease is lymphopenia with elevated neutrophil-to-lymphocyte ratio [1]. In search of the cause of lymphopenia, it may be pointed out the fact that SARS-CoV-2 easily infects human monocytes, macrophages and possibly dendritic cells (DCs), causing dysfunctions of these important antigen presenting cells (APCs) [2]. Observed DC dysfunctions facilitate improper antigen presentation, which obviously results T cell anergy, exhaustion and apoptosis [3], thus, may be contributing significantly in SARS-CoV-2 infection associated lymphopenia. Both helper (CD3⁺CD4⁺) and cytotoxic T (CD3⁺CD8⁺) cells were below normal range in subjects with Covid-19 keeping the T helper/suppressor ratio within normal limits [3].

Apart from the vaccine, some therapeutic measures are in deep search and ultimate remedy is awaiting. Global faith on natural remedy of any disease is increasing in face of its minimal collateral toxicities. In this context, India may offer their ancient vision with scientific justifications. We have acquired lessons from our research in last two decades with neem leaf glycoprotein (NLGP) (a water soluble component from neem leaves) to therapeutically immunomodulate murine hosts with cancerous diseases of different nature [4,5]. The neem tree has versatile

medical applications, thus, US National Academy of Science designated the tree as a ‘Tree solving global problems’ [6]. Like, COVID-19, lymphopenia along with increased neutrophil-to-lymphocyte ratio is a feature of cancerous diseases and chemotherapy worsen the situation creating threat to cancer management. In the dawn of our NLGP research, we found NLGP or its precursor, neem leaf preparation, can successfully overcome the problems of leucopenia and lymphopenia in murine tumor models, with or without chemotherapy [7,8].

Maintenance of M1 state of macrophages is not only crucial for cancer, it also appears effective in SARS-Cov-2 infection. NLGP treatment of murine melanoma bearing host demonstrated effective conversation from M2 associated TAM (CD11b⁺F4/80^{high}) to M1 phenotype (CD11b⁺F4/80^{low}) by regulating/suppressing STAT3-IL-10 mediated pathway. There was further downregulation of M2 markers including ManR, Ym1, and Fizz1 by NLGP [9]. Stage III supraglottic laryngeal tumor cell lysate (SLTCL) induced M2 TAMs polarized to M1 phenotype is also prevented by the intervention of NLGP [10] and higher anti-tumor functionality was observed. NLGP also restored the high IL-12 and low IL-10 level in M2 macrophages to their normalcy. Creation of M1 environment may help to combat Covid-19 infection too as M2 macrophage population (denoted by CD23⁺) was reported to be higher in severe Covid-19 cases. Moreover, coexpression of M2 marker (CD23) with M1 monocyte marker (CD38) was highest in severely affected patients [11]. NLGP might be effective to maintain M1 status of monocytes to offer better defense functions to Covid-19 sufferers.

We also established that NLGP can therapeutically restrict tumor

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growth in CD8⁺ T cell dependent manner [12]. Profound effect of NLGP on maintenance of CD8⁺ T cells was reported consistently [4,5]. In Covid-19 patients, remarkable decrease in CD8⁺ T cells is being observed in proportion to the severity of disease [3]. Patients those are released from hospital after recovery from severe infection have shown higher CD8⁺ T cell count in comparison to the same at the time of admission [13]. NLGP's effect on CD8⁺ T cell stimulation would definitely help in

SARS-CoV-2 infected individuals to get rescue from poor T cell count and functionality. What is the mechanism? Similar to the COVID-19 infected human system, microenvironment of cancer patients generates dysfunctional DCs having less expression of MHCs, co-stimulatory molecules- CD80, CD86, CD40 with higher expression of co-inhibitory molecule, PD1s on their surface [14]. NLGP has shown to normalize the dysfunctions of DCs obtained from cancer patients [15,16] or murine tumor models [17]. Accordingly, helps to present antigen effectively to CD8⁺ T cells to eradicate tumors [12,15–17] and to further restrain metastasis [5], by overruling their anergy, exhaustion and apoptosis [4]. These lessons, we believe, can be adopted in treatment of COVID-19 patients by improving functionality of DCs, thereby, offering more qualitative life to T cells to improve the lymphopenic condition. Such NLGP guided DCs might also be effective to generate proper SARS-CoV-2-specific effector and central memory T cells, as experienced from cancer system [18].

Asymptomatic SARS-CoV-2 infected individuals showed a potent and robust Type 1 immunity with high CD8⁺ T cell count, having a lower count of Th17 cells [19]. Conversely, severe COVID-19 patients presented with Th17-skewed immunity, fewer Type 1 responses and more antigen experienced T-cells. Several studies suggested that the Type 1 response was an independent protective factor for the prevention of hospitalization of severe forms of the disease [20]. NLGP is proven inducer of Type 1 immune response [21] having potent anti-tumor immunity. Actually, immune efficacy to combat tumors arises from the aggressive pro-inflammatory Th1 features of immunity. To promote such immunity, NLGP enhances the transcription factors, T-bet (Tbx21) [12,22] and eomesodermin (Tbr2) [23] within T cells, decreasing GATA3 [12] simultaneously. The consequent hike in IFN γ production alongside enhanced cytotoxicity of effector T cells, induce pro-inflammatory cytokines, IL-2 and IL-12 from the T cells [4] and the antigen presenting cells (APCs) [15] respectively. Suppression of GATA3 results in decreased secretion of the Type-2 cytokines, e.g., IL-4 from the T cells [4,24], thereby eliminating the possibility of interference from type 2 immune components. Moreover, systematic and phase wise activation/inhibition of TH1/TH2 response depending on the primary infection and secondary co-infections with SARS-CoV-2 opportunistic pathogens were reported [3]. NLGP as an immunomodulator can maintain the balance of TH1/TH2 immunity, which was frequently observed in cancer scenario [22,25] and expected to be effective in various stages of SARS-CoV-2 pathogenesis. Defects in type 1 immune responses [26] and excess type 2 immunity have both been shown to correlate with severe COVID-19 [20], suggesting that a maladapted adaptive immune response may potentiate disease progression. Induction of a predominant Th1 immune response by NLGP in the acute phase of the SARS-CoV-2 infection could be used as a useful cost-effective protective measure.

Increased levels of IL-1beta, IFN-gamma, IP-10, MCP1, IL-10, IL-17 have been reported in SARS-CoV-2 subjects [3]. Moreover, serum concentrations of IL-6 [20] and IL-2R [27] in Covid-19 patients are positively correlated to the severity of the disease. Thus, simultaneous elevation of pro- and anti-inflammatory cytokines is a common feature of SARS-CoV-2 infection and we experienced dual modulation of type-I/type-II immunity by NLGP in terms of downregulation of IL-6 (pro-inflammatory) and IL-10 (anti-inflammatory) [4,25; Das *et al*, *unpublished observation*] in several instances of our studies using cancer models. A well discussed phenomena is 'cytokine-strom' due to severe SARS-CoV-2 infection and manifested by dysregulated pro-inflammatory cytokine

milieu dominated by IL-6 [28], which is possibly playing crucial role in manifestation of Acute Respiratory Distress Syndrome or ARDS [29]. Such elevated IL-6 might serve as a critical factor to mediate mutually interrelated immune-suppressive loop among T cells, myeloid cells and others to exacerbate COVID-associated immunopathology [30]. Moreover, IL-6 may sensitize CD4⁺ T cells in such a way, which fails to help CD8⁺ T cells for its effector functions as well as memory generation desired to clear viral-infected cells and to protect host from second-time infection respectively [31]. IL-6 can mediate its downstream signal by two ways: in *cis*-signaling, it binds with IL-6R and complexed with gp130 and affect various immune cells, like, NK cells, macrophages, neutrophils, B and T cells of both innate and adoptive immune system, while in *trans*-signaling circulating IL-6 binds with soluble IL-6R and formed complex with gp130 and may activates many cells those even may not express IL-6Rs, like, endothelial cells. These both forms of IL-6 signals can activate downstream JAK/STAT3 signaling and contribute to develop cytokine release syndrome or CRS [32]. Notably, NLGP has shown its immune-modulatory activity towards dysregulated APCs (macrophages and DCs) to initiate proper antigen presentation to prime T cells by inhibiting the phosphorylation of STAT3 to initiate essential signaling, which coordinately helps to promote antigen-specific CD8⁺ T cell immunity to eradicate tumor cells [4,5,12,15,16]. Therefore, lesson from cancer research suggests NLGP may immunomodulate several SARS-CoV-2 infection-associated immune dysfunctions to control lymphopenia and others symptoms for viral clearance and life threatening 'CRS' by regulating 'cytokine milieu'.

CRediT authorship contribution statement

Anamika Bose: Conceptualization, Writing – review & editing.
Rathindranath Baral: Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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