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Potential role of GcMAF in suppressing the severity of COVID-19-induced immune responses: Lesson learned from HIV

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Introduction

Over the last six months, there have been increasing numbers of reports that struggle to understand the pathogenesis of the coronavirus disease 2019 (COVID-19) pandemic.

As of August 16, 2020, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been responsible for more than 21 294 000 infections and about 760 000 deaths worldwide [1], but the mechanisms of virus-induced host damage remain a mystery. Understanding the pathways behind viral pathogenicity just like cellular and tissue tropism, counteracting host defence processes and immunological responses are crucial to find new therapeutic strategies. Based on clinical reports, it is noteworthy that COVID-19 causes various degree of illness ranging from asymptomatic or milder symptomatic cases to severe lung injury or even multi-organ dysfunction with liver and kidney impairment [2–4]. Even in not severe patients, the heterogeneity of symptoms is consistent with the increasing evidence that SARS-CoV-2 shows a broad tissue tropism, being able to attack almost anything in the body [2–5]. To date, the most commonly investigated hypothesis about the underlying mechanisms of multi-organ failure may be summarized into three main targets: microcirculation dysfunction, overwhelming inflammation and abnormal coagulation [7]. Radiologic and laboratory findings as well as preliminary autopsy studies seem to support this hypothesis. The most common patterns seen on chest CT were ground-glass opacity, interlobular septal thickening, air bronchogram, bilateral patchy shadowing, crazy-paving pattern, and thickening of the adjacent pleura, resembling an interstitial involvement in viral pneumonia [2,3,7,8]. Under the light of microscope, the lungs revealed diffuse alveolar damage with formation of numerous hyaline membranes, very patchy and sparse interstitial chronic inflammation composed mainly of lymphocytes, thrombi within a few small pulmonary artery branches, congestion of alveolar septal capillaries, focal edema fluid, and macrophage infiltration within the airspaces [9–12]. The more significant laboratory abnormalities were metabolic acidosis, lymphocytopenia, leukopenia, thrombocytopenia, elevated levels of C-reactive protein (CRP), interleukin-6 (IL-6), lactate dehydrogenase (LDH) and D-dimer [2–7]. As firstly suggested by Huang C et al. [4], the systemic cytokine storm could play a key role in the virus-induced tissue damage. However, the question "what the link for the overproduction of pro-inflammatory mediators and the immune suppression, on the hand, and microvascular injury and thromboembolism, on the other, is", remains unclear.

Being the knowledge of this issue very scarce, lessons learned from other human pathogenic viruses, with specific reference to human immunodeficiency virus (HIV), could be diriment.

The hypothesis

Based on the aforementioned findings and on documented analogies between SARS-CoV-2 and HIV [13], we hypothesized that the reduced conversion activity of the Gc protein (human group-specific component (Gc)) into the macrophage activating factor (MAF) could have a key role in the dysregulate immune response induced by SARS-CoV-2, just like for HIV infected patients [14,15]. If this hypothesis is correct, it might help to set a valid strategy of immunotherapy also based on an off-label use of GcMAF in critically ill COVID-19 patients.

GcMAF and COVID-19: a literature review with a focus on the evaluation of the hypothesis

Gc globulin, DBP and GcMAF: three in one

Serum Gc protein, also known as vitamin D-binding protein (DBP), is a multifunctional protein present in plasma/serum at concentrations of 300–600 mg/L [16]. It carries a trisaccharide consisting of N-acetylgalactosamine with dibranched galactose and sialic acid termini at 420 threonine residue [17]. Stepwise hydrolysis of Gc protein by the inducible membranous β-galactosidase of stimulated B-lymphocytes, and by the Neu-1 sialidase of T-lymphocytes converts it into the active GcMAF [17–19]. On the contrary, deglycosilation of Gc protein by action of the enzyme alpha-*N*-acetylgalactosaminidase, named nagalase, secreted from HIV-infected cells leads to lack of macrophage activation and to immunosuppression, as a consequence [14,15]. It is remarkable

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that nagalase was demonstrated to be an intrinsic component not only of the envelope glycoproteins gp120 and gp160 of HIV but also of the hemagglutinin (HE) of influenza virus [15,20] and even produced by neoplastic cells [21–23]. Indeed, flu-like symptoms with serum nagalase activity similar to the influenza acute state were reported in the early stage of HIV-infection, so that the serum enzyme activity may be detectable at all phases of HIV-infection [14,15]. Similarly, most COVID-19 patients complained of flu-like symptoms in the early stages of the disease [2–5].

The role of GcMAF as a multifunctional immune modulator and possible implications in Covid-19.

It is now well known that DBP Gc-globulin plays a crucial role in immune system regulation as a primary defense against infections [14–20]. In addition to the storage and transport of active vitamin D3, GcMAF's effects include macrophage modulation, osteoclast activation, facilitation of neutrophil chemotaxis mediated by C5 derived peptide, superoxide activity, scavenging of circulating G-actin, anti-angiogenetic and anti-tumor properties [24–28]. Thus, this multifunctional protein, released into the blood stream, acts as a systemic immune modulator without pro-inflammatory activities. This means that any function impairment of Gc-globulin could result in a state of both immunosuppression and uncontrolled inflammation, just like in severe COVID-19. Interestingly, HIV viremia was associated with higher level of biomarkers of inflammation (measured by IL-6), monocyte activation (soluble CD14), and coagulation (D-dimer), leading to increased mortality, as compared with uninfected people [29]. Meanwhile, in COVID-19 patients, in addition to the reduced peripheral lymphocyte counts, mainly $CD4^+$ T and $CD8^+$ T cells, there were found significant high levels of pro-inflammatory cytokines and chemokines [2–7]. Indeed, GcMAF is not only a simple potent activator for macrophages, but more specifically is able to turn macrophage activity on at the sites of infection/inflammation and then to induce their apoptosis by upregulating caspase activity via the p38 and JNK1/2 pathways when no longer needed [30]. Post-mortem lung observations of patients died of COVID-19 showed the presence of mononuclear cells and macrophages infiltrating air spaces by autopsy [9–12].

With regards to the anti-oxidant properties, it was assessed that GcMAF promotes the superoxide generating capacity of activated macrophages and the production of nitric oxide (NO) [31]. An article by Nozik-Grayck et al. [32] pertinently and interestingly showed that the expression of extracellular superoxide dismutase (EC-SOD) mRNA and protein is cell- and tissue-specific and is prominent in lung, heart, blood vessels, placenta and kidney. In particular, high levels of EC-SOD are present in lung macrophages, alveolar type II cells, fibroblasts, vascular smooth muscle cells, and endothelial cells. EC-SOD limits oxidative stress and preserves NO bioactivity, thus protecting against a number of lung and cardiovascular diseases [32]. Even though only in a minority of cases, COVID-19 may progress to life-threatening complications, including respiratory failure, acute cardiac injury, acute kidney injury, septic shock, disseminated intra-vascular coagulation (DIC), and multi-organ dysfunction [2–5]. Hypoxemia was found to be associated with interstitial pneumonia and, in 10% to 20% of cases, developed into acute respiratory distress syndrome (ARDS) [2–5].

In this connection, it was documented that ARDS as well as organ dysfunction and septic shock is characterized by actin release which is involved in microvascular impairment [33,34].

DBP has an additional function in binding monomeric globular (G)-actin with high affinity. Thereby, rapidly removing polymeric actin fibrils from the blood stream, it prevents actin polymers from clogging the micro vessels not unlike fibrinogen/fibrin and consequently platelet aggregation and micro thrombi formation [26,35]. What we postulated could also explain hypercoagulability with elevated concentrations of D-dimer, fibrin degradation products increase, PT and aPTT prolongation, observed in COVID-19 patients [36,37]. Tang N et al. [37] reported that 71.4% of the non survivors of COVID-19 matched the grade of overt-DIC according to the International Society on Thrombosis and Haemostasis (ISTH) diagnostic criteria for DIC.

Murine models deficient in DBP showed lung damage caused by actin polymerization, developing severe acute lung inflammation with vascular leakage, hemorrhage and thickening of the vascular wall after actin injection [38]. Interestingly, the lung was the only organ that showed inflammatory injury after intravenous actin injection. The observed lung inflammation was consistent with alterations to lung microvascular endothelial cells. Indeed, when lung endothelial cells were exposed to DBP-actin complexes in vitro showed enhanced cell death [38]. Reduced levels of DBP were even observed in sepsis and organ dysfunction of trauma patients as well as complete depletion of free DBP in those affected by septic shock [33,34]. These data could provide support for pathogenic explanations of cellular and tissue damage by SARS-CoV-2 and, at the same time, for the therapeutic use of DBP to bind extracellular actin and counteract microcirculatory alterations.

Whereas DBP also binds free fatty acids, it was shown that the administration of GcMAF complexed with oleic acid (OA) via nebulisation or subcutaneous injection led to rapid decrease of blood pressure and increase in splenic blood flow, as a result of a verisimilar synergistic NO release by OA-GcMAF-activated alveolar and splenic macrophages [31]. Severe or critically ill COVID-19 patients developed clinical typical manifestations of shock, even in the absence of overt hypotension [7].

Furthermore, it was found that GcMAF can inhibit the angiogenesis induced by pro-inflammatory prostaglandin E1 [39], which serves roles in the promotion of vascular endothelial growth factor (VEGF) expression [40]. A key role of VEGF in acute lung injury and ARDS was confirmed [41].

Explaining the clinical heterogeneity of Covid-19 with DBP polymorphisms, estrogens and vitamin D

Reflecting the fact that clinical features and severity of symptoms vary widely between and within each COVID-19 patient, with older males more likely to be affected and in a more severe manner [42], we sought to relate it with some special feature of DBP. Several studies showed that the polymorphisms of DBP were associated with susceptibility or resistance to disease states including chronic obstructive pulmonary disease [43,44]. Moreover, whereas androgens were not found to have any effect on circulating levels of DBP, exposure to high levels of estrogens increased them by up to 50%, suggesting a potential protective role of estrogens against COVID-19 [28]. On the other hand, in relation to vitamin D status, advanced age was recognized as one of the major risk factors for vitamin D deficiency [45]. Animal-based studies also demonstrated that deficiencies in both dietary protein- and energy-intake decreased the concentration of DBP in the circulation [46]. These data seem to be in line with the growing evidence that vitamin D supplementation could reduce the risk of COVID-19 infections and deaths [47–49].

Towards the application of GcMAF as immunotherapy: from cancer to SARS-CoV-2 infection

To date, a pharmaceutical grade GcMAF agent that can be administered to patients with COVID-19 is not developed yet, but, as our hypothesis suggests that GcMAF, an activator of macrophage, a key player of innate immunity, may be effective in suppressing the severity of COVID-19-induced immune responses, it would be advisable to proceed in this way.

In the GcMAF development timeline, there have been three major types of GcMAF until now: purified (i.e. first-generation) GcMAF, serum (i.e. second-generation) GcMAF, and oral colostrum MAF (i.e. third-generation GcMAF).

The first-generation GcMAF is produced from Gc protein isolated from human serum by an artificial enzymatic method using an affinity

column modified with 25-hydroxy-vitamin D3 [50].

The second-generation GcMAF is prepared from degalactosylated/desialylated human serum without isolation of Gc protein using vitamin D affinity chromatography, leading to a higher concentration, stability and activity of the final GcMAF, without the risk of cross-contamination between different serum samples [51]. In addition, the second-generation GcMAF has been shown to have increasing activity of macrophages, superoxide radical generation, anti-angiogenetic effects, and antitumor effects.

In 2014, Saisei Mirai clinics (cell processing center, clinic in Kobe, Osaka and Tokyo), in collaboration with Tokushima University, developed a new form of GcMAF made from bovine colostrum [52]. Colostrum MAF has the advantage that it can be orally administered, namely, in an acid-resistant enteric capsule to activate macrophages in the gut-associated lymphoid tissue (GALT) [53]. This is considered to be the largest macrophage pool in the body playing a very important role in maintaining and regulating mucosal immunity [53]. Macrophages in the gastrointestinal mucosa also could modulate the respiratory tract mucosal immunity through immune regulation, the so-called “gut-lung axis”. Additionally, colostrum MAF is administered as a powder in the mouth to activate macrophages in the lymphoid tissue of the Waldeyer's tonsillar ring. In this regard, it is remarkable that Angiotensin Converting Enzyme 2 (ACE2), an Entry Receptor for SARS-CoV-2, was found to be highly expressed in gastrointestinal epithelial cells, providing a prerequisite for SARS-CoV-2 infection [54]. On this base, if we consider GcMAF as an off-label immunomodulating agent for the treatment of COVID-19, oral administration should be preferred to all the other ones.

Although the administration of GcMAF is a yet an unapproved therapy, data from previous studies and clinical practice reported its effectiveness in the treatment of many pathologies such as HIV infection [55] and other infectious diseases [53], some types of cancer [56–60], juvenile osteopetrosis [61], immunological (systemic erythematous lupus) [62] and neurological (multiple sclerosis, autism) diseases [57,63,64]. In the same conditions, it was found an inverse correlation between the MAF precursor activity and serum levels of nagalase (reference ranges from 0,32 to 0,65–0,95 nM/min/ng), therefore showing to be other than pathogenicity or cancer biomarkers, also good prognosticators of illness and response to therapy [54–64].

However, it’s clear that only well-designed clinical trials will be able to properly evaluate the therapeutic use of GcMAF.

The main targets of the pharmacologic approaches to COVID-19, especially for the complicated cases, are addressed to modulate the immune system and counteract the overwhelming inflammation.

Notably, the mechanisms we have hypothesized about the possible pathogenesis of the cell and tissue damage induced by SARS-CoV-2 seem to provide a common denominator in explaining the effects of most drugs currently considered for the treatment of COVID-19: these include antivirals (i.e. remdesivir, lopinavir/ritonavir, darunavir, cobicistat) and immunomodulating and/or anti-inflammatory drugs [65,66].

In particular, based on their antiviral activity [67], chloroquine and hydroxychloroquine, initially conceived as antimalarial therapeutics, were proposed to treat patients hospitalized with COVID-19, better if associated to azithromycin, showing promising efficacy in “inhibiting the exacerbation of pneumonia, improving lung imaging findings, promoting a virus negative conversion and shortening the disease course“ [68,69]. On the other hand, hydroxychloroquine is the cornerstone of medical therapy in lupus, where it acts as an immunomodulatory without immunosuppressive effects [70]. However, in light of ongoing serious cardiac adverse events [71] and other serious side effects, the known and potential benefits of chloroquine and hydroxychloroquine no longer outweigh the known and potential risks for the authorized use in COVID-19 patients. In addition, the COVID-19 Treatment Guidelines Panel recommends against using hydroxychloroquine plus azithromycin for the treatment of COVID-19, except in a clinical trial [72].

Tocilizumab, an IL-6 antagonist, approved for the treatment of rheumatoid arthritis and juvenile idiopathic arthritis, also had therapeutic application in critical COVID-19 patients, providing encouraging results [73]. However, the phase III clinical trial (COVACTA) [74] for evaluating tocilizumab in hospitalized patients with severe COVID-19 pneumonia found no difference between tocilizumab versus placebo in intensive care requirements or mortality. Therefore, there are insufficient data for the Panel to recommend either for or against the use of the IL-6 inhibitors for the treatment of COVID-19 [72].

The rationale basis for the use of monoclonal antibodies in patients affected by SARS-CoV-2 seems to lie in the so-called systemic cytokine storm. Taking into account the key role of VEGF in enhancing angiogenesis in acute lung injury and ARDS [75], two trials, evaluating the efficacy of bevacizumab as VEGF antagonist in the treatment of COVID-19 (BEST-PC and BEST-RCT), were started [76,77].

Possible consequences of the hypothesis and conclusion

Less than two months after the declaration of pandemic state by the World Health Organization, every effort by the entire scientific community has been made to face this worldwide emergency of COVID-19 in the best possible way.

However, at the present days, the underlying mechanisms of pathophysiology remain unknown. Nowadays, although a number of preliminary clinical trials are underway and scientific evidence is growing on this topic, neither specific drugs nor effective preventive measures are yet available for the treatment of COVID-19. Anyway, it seems we still have a great deal of work to find the “miracle care”. However, due to the risk of serious drug-related adverse events, the immunomodulatory and anti-inflammatory drugs currently used for COVID-19, are still restricted to carefully selected and complicated cases [71,78–81].

So, in sight of this, given its multifunctional properties, we believe that GcMAF could have a very important role in the pathophysiology of organ damage induced by SARS-CoV-2, providing explanations which are consistent with the clinical, radiological and histopathological findings observed in patients with COVID-19.

Despite burgeoning data from case series of various pathological conditions demonstrated the potential clinical benefits of GcMAF as above mentioned [55-64], no randomized controlled clinical trials verifying these preliminary results have been made till now. So, there are still unresolved controversies about the possibility of an its therapeutic application. To date, no other researcher has investigated the possibility of a potential linkage between GcMAF and COVID-19.

However, in view of the immunomodulatory potential and the high safety profile of GcMAF and because COVID-19 remains a life-threatening condition in many cases, despite currently recommended therapies, we think it is worth exploring our hypothesis further by:

- detecting the MAF precursor activity of serum Gc protein and serum Nagalase activity in all COVID-19 patients;
- providing in a short time, in addition to currently used drugs, GcMAF to critically ill COVID-19 patients, requesting Institutional Review Board (IRB) approval for compassionate use protocols on an emergency basis;
- confirming, in prospective randomized controlled clinical trials, the efficacy and the safety of the proposed treatment, in order to allow the international scientific community to analyse the results and guide the future clinical practice.

According to the provided literature overview, we firmly believe that GcMAF deserves be tested as immune-therapeutic to increase macrophages functionality for earlier SARS-CoV-2 viral control, protection against COVID-19 progression by limiting epithelial damage, control local inflammation and prevent from the hyperinflammatory

immune response. For this purpose, we planned a Phase-II interventional clinical trial evaluating the effectiveness and safety of Oral immunotherapy with Third Generation GcMAF in hospitalized patients with COVID-19 pneumonia (COral-MAF1 Trial) at the "Ospedale del Mare" Hospital, Naples, Italy. For the time being, we are waiting for clinical trial approval by local ethics Committee.

In conclusion, even though our hypothesis might be not fully correct, we still wanted to give our own contribution to the research on COVID-19 pandemic which had the only underserved merit to give all humans across the world a common goal.

So, as we still see life from a rosy perspective from this small Italian hospital facing the sea, our last thought and gratitude are for all the researchers and health care professionals which are committed to the forefront of the war against SARS-CoV-2.

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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