

Rationales of GcMAF as a therapeutic target for SARS-CoV-2

The fact that around 96% of patients recover from the infection despite there being no antiviral therapeutics that specifically target human coronaviruses suggests that the human immune system has mechanisms to combat SARS-CoV2.

What is GcMAF?

GcMAF is activating macrophages lymphokines, its effects are seen starting from the 10ng /ml dose. GcMAF or Gc protein-derived macrophage activating factor is a protein that resulted in sequential deglycosylation of his precursor - the vitamin D-binding protein.

What is macrophage subtype targeting by GcMAF?

Due to differences in GcMAF, clinical effects depend on the pathological condition, type and stage, suggesting that its mode of action is different from other macrophage activation cytokines such as interferon- γ (IFN- γ), which specifically targets M1 macrophages. GcMAF interactions are not restricted to any specific phenotype only. Macrophages are highly plastic cells, their transcriptional profiles supposed to be highly responsive to the GcMAF stimuli, all range of phenotypes that are often grouped as M1, M2a, M2b and M2c types could in different level respond to make an activated phenotypes combination better adapted to presented challenges. Quite possible that initially M1 phenotype is a most responding one, and its could polarize on M2 soon after activation when infectious pathogens concentration decreased in the resident tissue.

On our clinical observation, the following GcMAF effects were seen. Regression of inflammatory clinical and biochemical markers were observed in patients with rheumatoid arthritis. The stable improvement of neurocognitive functions, attention and behavior under GcMAF treatment on autism spectrum disorders which likely are results of turn to the relatively anti-inflammatory macrophages phenotype associated with these conditions the proinflammatory state of microglia, as macrophages are plastic cells and may transition between different states. GcMAF has also shown efficiency in the prevention of clinical symptoms onset when taken on the prodromal period of influenza.

Targeting alveolar macrophages

In murine models of pulmonary infection alveolar macrophages clear pathogens up to a defined threshold without overt features of pneumonia, but when alveolar macrophages fail to control these subclinical infections recruitment of inflammatory cells, predominantly neutrophils, is required to control infection [Dockrell DH et al. 2003]. It was also demonstrated that mice with decreased numbers of alveolar macrophages, or with alveolar macrophages of reduced microbicidal capacity, are more susceptible to develop pneumonia and that the threshold inoculum required to generate pneumonia is reduced significantly [Dockrell DH et al. 2003].

However, alveolar macrophages are very long-lived cells that may live for prolonged periods in steady-state [Murphy J *et al.* 2008]. GcMAF could be a prophylactic/preventive therapeutic for pre-activation of macrophages, making them polarized from steady-state to functional alert state when there is a high risk of exposure to COVID-19.

Alveolar macrophages may demonstrate simultaneously both M1 and M2 characteristics during acute inflammation in the lungs. Their ability to generate inflammatory responses is regulated tightly to ensure that lung injury is controlled. M2 activated alveolar macrophages are ensuring tissue remodeling and repair, they are characterized by high levels of IL-10, and scavenger receptors expression and exert anti-inflammatory regulation. As mentioned above the initially M1 phenotype is most likely responding to GcMAF stimuli, which is supported by *in vitro* data of enhanced phagocytosis. M1 phenotype could polarize on M2 after activation when pathogen concentration decreased in the resident tissue. The subjective evidence for boosted M2 macrophages phenotype functionality are based on our animal studies, showing decreasing anti-inflammatory cytokines in blood serum after 8 days of GcMAF treatment. The observed remission of chronic obstructive pulmonary diseases under GcMAF intake is supporting the statement of enhanced M2 phenotype functionality. There are also other data where GcMAF has been reported to normalize observed dysregulation of gene expression of the endocannabinoid system in autism and to down-regulate the over-activation of bone marrow-derived macrophage from autistic children [6]. The GcMAF effects on connective tissue remodeling [4] and repairing supports its anti-inflammatory actions.

Both phenotypes are important GcMAF's targets for controlling systemic and lung inflammation and for the prevention of acute respiratory distress syndrome in COVID-19 pneumonia.

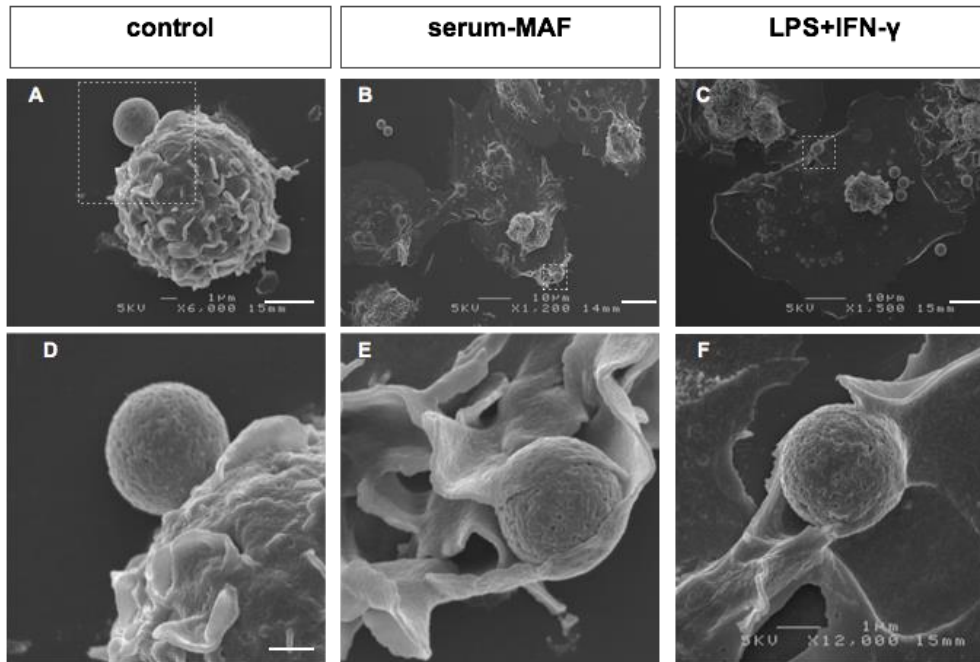
Reconstitution of gaps in the innate immune response to SARS-CoV-2

In recent studies, the very rapid phagocytic activation under Saisei Mirai serum GcMAF treatment was shown. It has also been shown to increase antigen internalization 3-fold compared to LPS+IFN- γ on the macrophage cell line. There is a link to a short video of serum GcMAF induced macrophages membrane restructuring which happens within 5 min treatment with GcMAF, the filmed phagocytosis also looks higher compared to the control: <https://lt.saisei-eu.com/lhp/wp-content/uploads/vid/mv-ssi-2020.mp4>

In the video and figures 1, 2, 3, 4 presented data from the following publications:

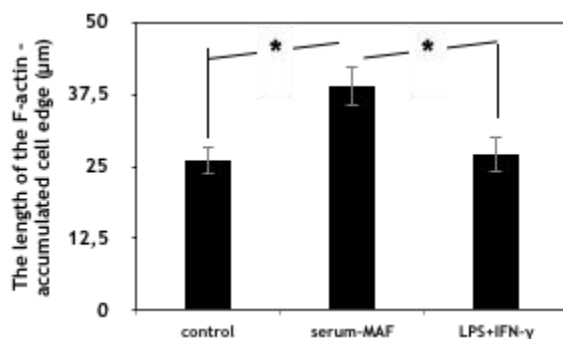
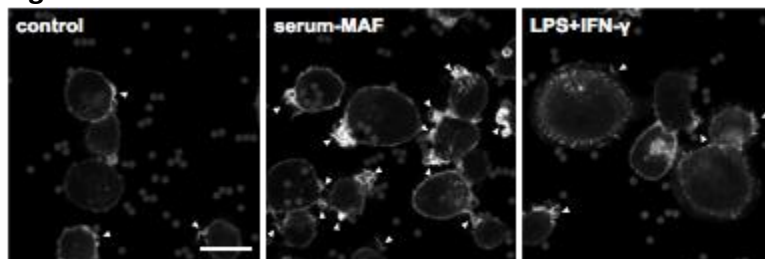
1. Kawakatsu K, Nishikata T *et al.* Characteristic Morphological Changes and Rapid Actin Accumulation in Serum-MAF-treated Macrophages. *Anticancer Res.* 2019.
2. Mami Ishikawa *et al.* A high-throughput quantitative assay system for macrophage phagocytic activity. *Macrophage* 2018.

Figure 1



Morphological changes of THP-1-derived macrophages following MAF treatments. After a 4-h treatment with 8.17 $\mu\text{g/ml}$ serum-MAF (B, E), 100 pg/ml LPS + 20 ng/ml IFN- γ (C, F), and control (A, D), beads were added to macrophages and fixed for SEM observation. Low magnification, whole cell images (A-C) and enlargements of the engulfing region (white rectangle) are shown (D-F). Scale bars; 10 μm in A-C, 1 μm in D-F.

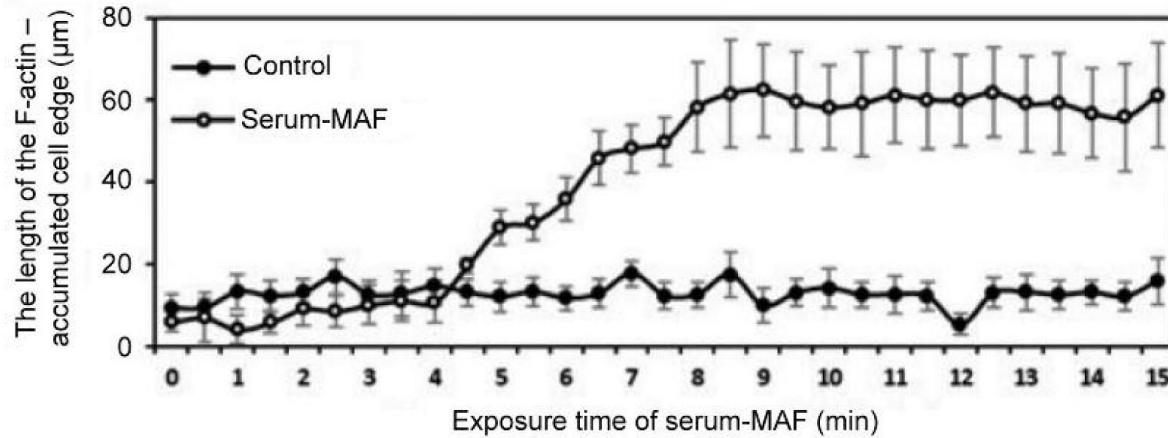
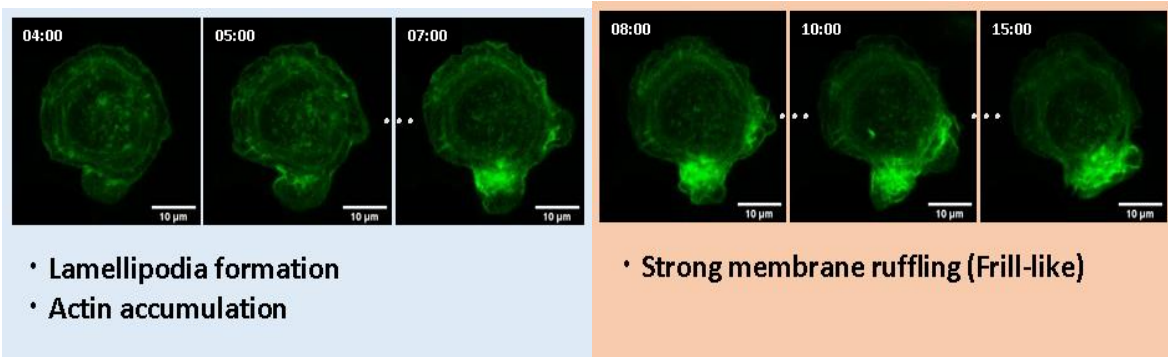
Figure 2



Differences in actin accumulation at the edge of the cells. Confocal images of Lifeact-THP-1 derived macrophages, treated with 8.17 $\mu\text{g/ml}$ serum-MAF (B), 100 pg/ml LPS + 20 ng/ml IFN- γ

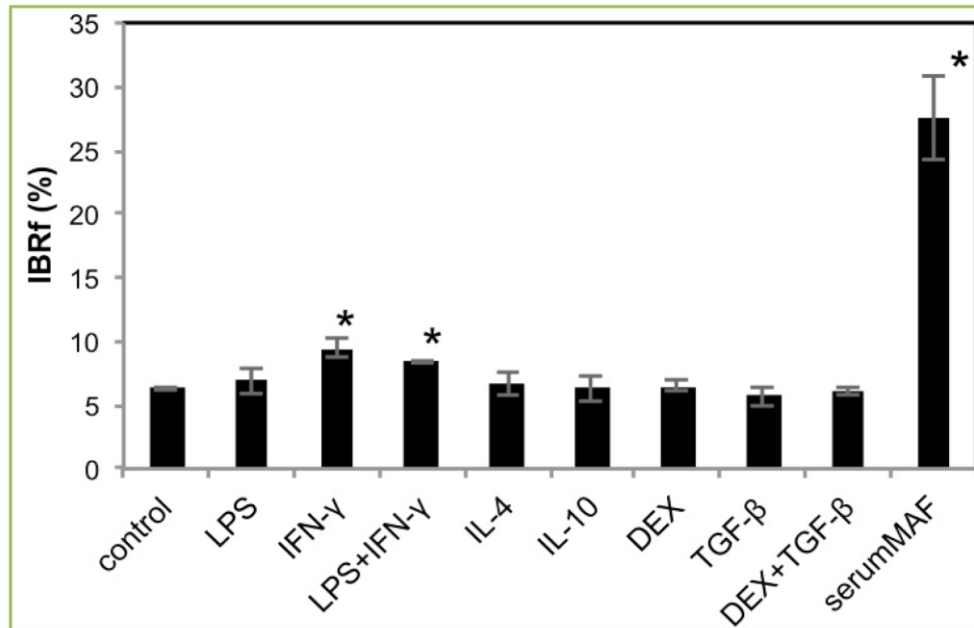
(C), or without MAF (A) are shown. White arrowheads indicate actin accumulations in lamellipodia tips. Three-dimensional analysis of serum-MAF activated macrophage (D) represents intricate membrane ruffling at the site of actin accumulation. Scale bar; 10 μm . Actin accumulation was quantitatively analyzed using these images (E).

Figure 3



Time lapse images of Lfeact-THP-1 derived macrophages after the start of treatment with serum MAF compared to control are indicated in the graph. Actin accumulation was quantitatively analyzed.

Figure 4. The MAFs activated macrophages phagocytic activity comparison [Mami Ishikawa *et al.* 2018]



The virus replication cycle of SARS-CoV is completed in approximately 6 h [Ng *et al.*, 2003], the virus simply appears to replicate to high titres well before type I IFNs are induced. In a microarray analysis of the cellular gene expression of SARS-CoV-infected human macrophages, SARS-CoV failed to induce significant IFN- α/β gene expression [Cheung CY, *et al.*, 2005]. SARS-CoV has developed mechanisms to induce a delayed response of the innate immune system in both 293 fibroblast cells and DCs, which allows the production of infectious progeny virus in both cell types [Spiegel M *et al.* 2006].

Classical activation of M1 macrophage polarization requires IFN- γ in combination with TLR4 signaling and is characterized by increased expression of pro-inflammatory mediators and effectors enabling phagocytosis and killing of pathogens. However, GcMAF is itself shown to induce sustained activation on Lifact-THP-1 derived macrophages seen after 5 min of treatment (Figure 3). Such a rapid macrophage activation followed by the fast phagocytic membrane's structure remodeling (Figure 1, 2) and 3-fold over antigen internalization compare to the most potent known macrophage activators combination LPS+IFN- γ (Figure 4) are for the first time reported.

It is expected that administration of GcMAF in humans during the very early stage of SARS-CoV-2 infection induces the similar to observed on *in-vitro* pre-activation of mucosal alveolar macrophages, which initially have to accomplish with type I IFNs synthesis upregulation. This could be the way to overcome the SARS-CoV mechanism of replication via suppression of the IFN- α/β response in host macrophages with innate immune response delay.

SARS-CoV infections of macrophages lead to the initiation of viral gene transcription and viral protein synthesis, but no infectious virus produced, and hence SARS-CoV infections of human macrophages appeared to be abortive [Chung Y. Cheung, *et al.* 2005]. In this connection,

GcMAF's ability to strongly enhance the **phagocytosis** of SARS-CoV viral particles by macrophages on the background of early sufficient IFN- α/β response is a chance to increase the probability of the SARS-CoV abortive infection course. Even viral replication restriction is crucial, as uncontrolled by innate immunity, it leads to progressive increases in the viral load, which in turn drives the systemic hyperactive proinflammatory response - the dramatic point of SARS-CoV disease responsible for the clinical severity and mortality.

Ags processing

In a variety of in-vitro assays was shown that GcMAF induced phagocytosis accomplished with antigens processing. The lysing activity as judged by reduction of pH and transition into phagolysosome or lysosome and release of reactive oxygen and reactive nitrogen molecules have been strongly enhanced by GcMAF [12-14]. It could lead to antigens presentation to lymphocytes on the early stage of SARS-CoV-2 infection when these cells' functionality and numbers are not yet affected by COVID disease and prompt the adaptive immunity involving and antibody formation process.

From the perspective view, apart from the antiviral activity, GcMAF seems to be a perfect adjuvant to the SARS-CoV-2 vaccine too.

Impaired GcMAF synthesis

GcMAF macrophage activation pathway is required of membranous glycosidases of inflammation-primed lymphocytes which rapidly convert serum Gc protein to the potent macrophage-activating factor. This inflammation-primed macrophage-activation process appears to be the major macrophage-activation cascade. A defect in the inducible β -galactosidase of B lymphocytes in the macrophage-activation cascade produces dysfunctional macrophages (Encyclopedia of Immunology, 1998).

It was shown that SARS-CoV could not only infect lymphocytes but also replicate in them. Lymphopenia was detected in 63% of hospitalized patients with pneumonia. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood are reported [Zheng, H *et al.*2020]. All the above affecting the conversion of Gc protein to GcMAF due to the deficiency of membranous glycosidases secreted by T and B lymphocytes.

Exogenous GcMAF therapy can bypass the defective lymphocyte function and act directly on phagocytes, and most importantly that it allows initiative controlled by adaptive immunity GcMAF's macrophages activation pathway in the few days earlier be the required lymphocytes activation will naturally take a place during infection course.

Conclusion

Activating macrophages in the respiratory tract with GcMAF can be the key to a more rapid and efficient innate immune response against COVID-19, which is pivotal in a condition without

pre-existing immunity in the population. Absolute safety and targeting crucial points of COVID-19 infection as viral replication control and prevention of pathological hyperimmune response make GcMAF a compelling candidate for urgent clinical trial implementation.