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Case Report

Severe SARS-CoV-2 infection treated with the mannose binding lectin associated serine protease 2 (MASP2) inhibitor Narsoplimab

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Abstract

In SARS-CoV-2 infection, increased inflammation, complement activation, and excessive clotting are responsible for morbidity and mortality. Recent reports suggest that mannose binding lectin (MBL) and mannose-associated serine protease 2 (MASP2) lies at the intersection of these pathways. Consistent with this concept, we observed that the SARS-CoV-2 spike protein binds MBL-MASP1/2 complex in human serum. We therefore suggested treating a severely ill, ventilated SARS-CoV-2 patient in whom all other treatments had failed with the anti-MASP2 antibody Narsoplimab. Following a 4-week course of Narsoplimab, the patient made a near complete recovery, supporting the utility of MASP2 inhibition for treating hospitalized SARS-CoV-2 patients.

Keywords: SARS-CoV-2, Narsoplimab, COVID-19, MBL, MASP2

Introduction

The COVID-19 pandemic caused by SARS-CoV-2 has exerted a horrific toll on human health, both directly via millions of deaths and indirectly through the massive economic and societal damage accompanying worldwide lockdowns [1]. While successful vaccines have been developed, it will be years before the majority of the world's population can be vaccinated, a process made more challenging by emerging variants [2]. In the meantime, the virus presents a tremendous challenge to the health care system due to its contagiousness and unpredictability, with individuals at one extreme experiencing minor symptoms while those at the other extreme suffer severe illness and/or death [1]. A central problem has been understanding the mechanisms through which the virus results in such grave illness in certain patients.

Several studies have suggested that the virus causes critical illness by inducing excessive inflammation, activating complement, and initiating the clotting cascade [3-6]. These pathways intersect at the lectin complement pathway, which is initiated with mannose binding lectin (MBL) and the mannose-associated serine proteases 1 and 2 (MASP1 and MASP2). Activation of this pathway leads to tissue complement deposition and production of the pro-inflammatory molecules C3a and C5a [7,8]. MASP2 also directly activates the clotting cascade by cleaving prothrombin [9-11]. Importantly, the anti-MASP2 human monoclonal antibody Narsoplimab (being developed by Omeros) was recently shown in an open-label study involving six Italian SARS-CoV-2 patients requiring mechanical ventilation to have strikingly positive effects, with all patients being discharged from their hospital and making almost full recoveries [12].

In light of these observations, we investigated the binding of the SARS-CoV-2 spike protein to circulating human proteins and found that the spike protein specifically bound the MBL-MASP1/2 complex in human serum, thus suggesting that the virus may directly activate MASP2. As a result, in a hospitalized, ventilated COVID-19 patent in whom all other treatment options had failed, we suggested administering Narsoplimab. Remarkably, after receiving a 4-week course of therapy, the patient went on to make a nearly complete recovery, indicating that MASP2 inhibition (and thus lectin complement pathway inhibition) with Narsoplimab may be a viable treatment option for hospitalized SARS-CoV-2 patients.

Materials & Methods

SARS-CoV-2 spike protein binding to human serum proteins

A recombinant protein composed of the extracellular domain of SARS-CoV-2 spike protein (Wuhan-Hu-1 strain) in which the furin cleavage site was mutated (R685A) was coated onto 96-well plates (3 μg/well). Plates were washed and blocked with 1% BSA. Sera and plasma from 5 donors were diluted in half with PBS, with 100 μL added to wells and incubated overnight at 4°C. After aspiration, plates were washed, and 100 μL of 1% acetic acid were added. Eluates were transferred to PCR plates and dried under nitrogen. Subsequently, 100 μL of 50% acetonitrile, 1% iodoethanol, and 0.25% triethylphosphine in 50 mM ammonium carbonate were added to the samples for 1-hour at 37°C. After nitrogen-drying, 100 μL of 5 μg/mL trypsin in 100 mM ammonium bicarbonate were added at 37°C overnight. Digested samples were desalted using Ziptips prewetted with 3 aspirate/dispense cycles of 50% acetonitrile and 0.1% TFA and equilibrated with rinses of 1% acetonitrile and 0.1% TFA. Peptides were captured by 20 aspirate/dispense cycles, washed, and eluted with 50% acetonitrile and 0.1% TFA. Eluates were nitrogen-dried and reconstituted in 10 μL of 1% acetonitrile and 0.1% TFA.

Mass spectrometry peptide analysis

Samples were analyzed with a Thermo Q-Exactive mass spectrometer using a Thermo-Easy liquid chromatograph-high performance liquid chromatography system. Peptides were separated using a 75 μm C18 column interfaced to a custom nano-spray interface with electrospray potential of +1.2 kV and a capillary temperature of 200⁰C. Solvents were A 0.1% formic acid and B 80% acetonitrile and 0.1% formic acid. The gradient was 35-min using a flow rate of 250 nL/min, starting with 3-min 5-20% B ramp, 29-min 20-60% B ramp, 1-min 60-95% B ramp, and 2-min hold at 95% B. The instrument was run from 300-2000 m/z at 120,000 resolution (maximum injection time of 50-ms). Data-dependent fragmentation was performed on the top 20 2+, 3+, or 4+ ions at a collision energy of 25 and scan resolution of 30,000. Peptides were identified by searching spectra against a Uniprot proteomics database [13]. The fixed modification was iodoethanoylation of cysteines. Variable modifications were oxidation of methionines and deamidation of asparagines/glutamines. Identification filters were 10 ppm for the parent, false discovery rate of 10%, and at least 2 peptides/protein matching. Peptides were integrated with a 1-m/z radius and 2-min window. Mean peptide areas were determined for each donor, with overall 5-donor means calculated using JMP software version 15.

Case Description

The patient was a 53-year-old white male with a past medical history of asthma, hypertension, hyperlipidemia, gastroesophageal reflux disease, and hypersensitivity lung disease who presented in respiratory distress with a fever, headache, and positive COVID-19 test. He was initially prescribed an inhaler and steroids by his primary care physician, but his condition deteriorated. Following hospital admission, he developed rapidly worsening symptoms and required supplemental oxygen. He was given remdemesvir, dexamethasone, and convalescent plasma along with supportive therapy but continued to decline. He required more oxygen and was transferred to the intensive care unit, where he was placed on

a ventilator and continued on a downhill course for another week. After all treatment options had failed, the anti-MASP2 monoclonal antibody Narsoplimab was administered intravenously at 4 mg/kg twice a week for 4 weeks.

Results

SARS-CoV-2 spike protein binding to MBL-MASP1/2 complex in human serum

To determine which circulating human proteins bound SARS-CoV-2 spike protein, we incubated solid phase-coupled recombinant spike protein with human serum, EDTA plasma, or heparin plasma from 5 donors. After washing away unbound proteins, bound proteins were digested into peptides, and mass spectrometry was used to identify the peptides and their corresponding human serum proteins. Figure 1 shows the results from these experiments. Proteins consistently identified from each donor were MBL, MASP1, and MASP2. Interestingly, enrichment of each of these was only observed with serum or heparin plasma. No binding above that of the control was observed with EDTA plasma, indicating that binding of these proteins to SARS-CoV-2 spike protein was calcium-dependent. In addition, the consistent binding of all three proteins suggested that MBL, MASP1, and MASP2 circulate in an MBL-MASP1/2 complex in human serum, a concept consistent with evolving thinking about the MBL-lectin complement pathway [7-11].

Figure 2 shows a proposed model for how SARS-CoV-2 activates the MBL-lectin complement pathway and the clotting cascade. High mannose glycans present on the SARS-CoV-2 spike protein bind to circulating MBL-MASP1/2 complex [14,15]. Following spike protein binding to MBL present in the complex, MASP1 is autocatalytically activated via structural rearrangement, leading to cleavage and activation of MASP2 [16]. Active MASP2 can then cleave prothrombin, C2, and C4, thus both activating the clotting cascade and causing complement activation that leads to increased inflammation (via C3a and C5a) and membrane attack complex (MAC) deposition [7-11]. Endothelial cell damage caused by the MAC results in the release of damage-associated molecule pattern (DAMP) molecules that are then recognized by ficolin/MASP complexes in a similar manner to MBL-MASP1/2, with the result being an uncontrolled positive feedback loop that can then cause even more clotting, inflammation, and complement deposition [17]. After considering this possible model, we suggested treating a severely ill, hospitalized SARS-CoV-2 patient with the anti-MASP2 human monoclonal antibody Narsoplimab [12].

Clinical course of SARS-CoV-2 patient

After obtaining FDA and hospital IRB permission for Narsoplimab's compassionate use and Narsoplimab itself as a generous gift from Omeros, the patient was started on a four-week course of Narsoplimab at 4 mg/kg twice a week. For each dose given, Narsoplimab was added to 50 ml of D5W and administered intravenously over 30 minutes. After the third dose, the patient began to show improvement. By the fourth dose, improvement became more evident, with the patient being able to sit up, eat ice chips, and go off the ventilator for 4-hours at a time. After the fifth dose, the patient was able to complete a 24-hour trial off the ventilator and regained his ability to speak. The patient was then able to be removed from the ventilator. After the sixth dose, the patient was transferred from the intensive care unit to a step-down
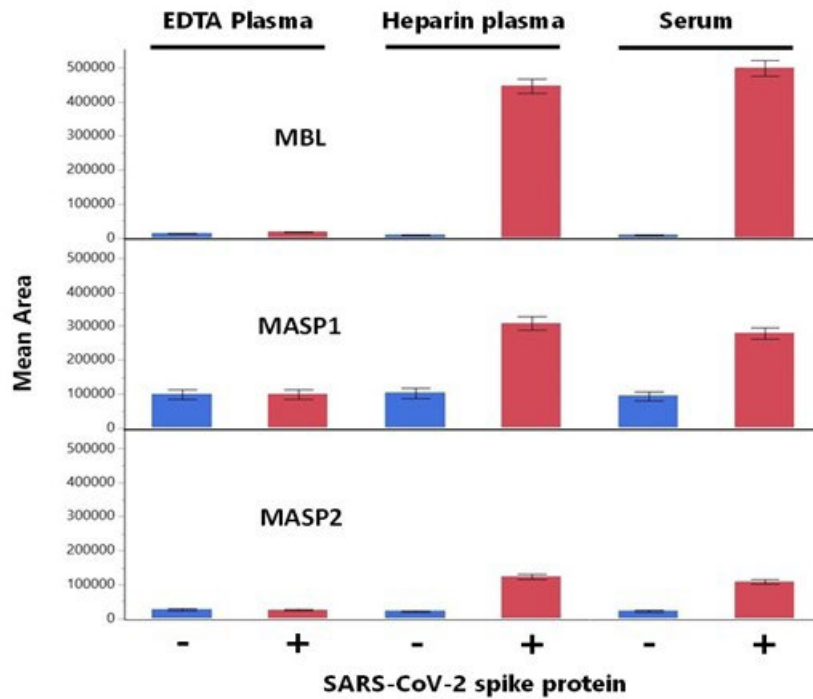
Figure 1: SARS-CoV-2 spike protein binding of circulating MBL-MASP1/2. Serum, heparin plasma, and EDTA plasma from 5 donors were incubated with SARS-CoV-2 spike protein or a negative control (bovine serum albumin). Bound proteins were identified via mass spectrometry. MBL, MASP1, and MASP2 were consistently identified as enriched, bound proteins. The mean peptide areas from each donor for each protein are plotted. The negative EDTA plasma result confirms the calcium-dependency of MBL-MASP1/2 binding. Results shown are the mean peptide area ± SEM.
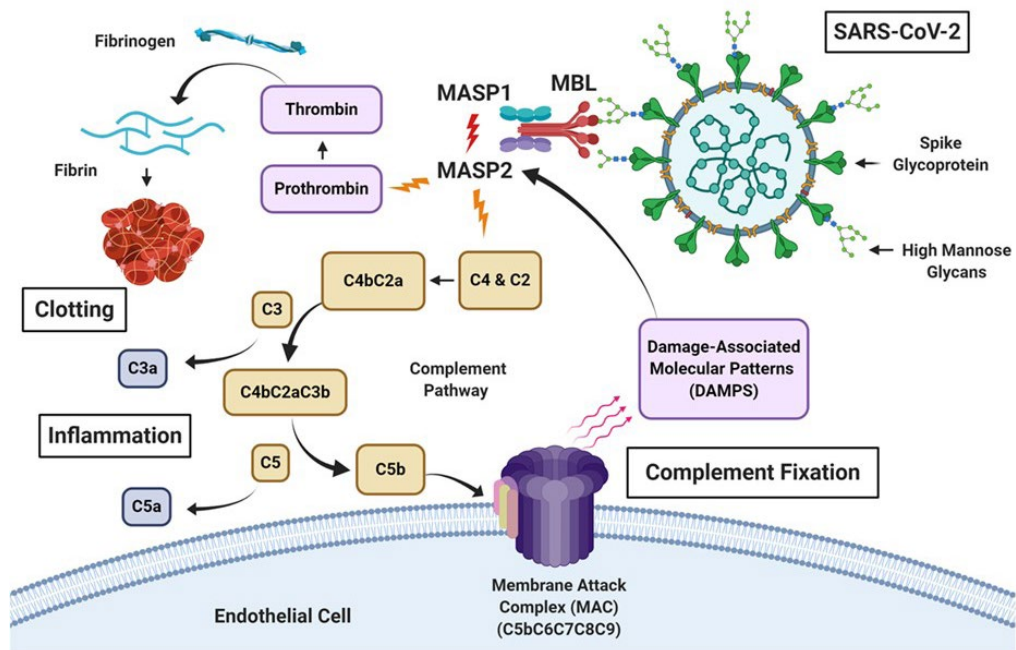

Figure 2: A model for SARS-CoV-2 activation of the clotting and lectin complement pathways. The MBL-MASP1/2 complex binds to high mannose glycans on the SARS-CoV-2 spike glycoprotein. After the complex binds, MASP1 is autocatalytically activated and cleaves/activates MASP2. Active MASP2 cleaves prothrombin, C2, and C4. Thrombin activation leads to clotting, while cleavage of C2 and C4 initiates the complement pathway. Deposited MACs release endothelial cell damage-associated molecule pattern (DAMP) molecules that are further recognized by ficolin/MASP complexes, resulting in a continuous positive feedback loop.

unit on 35% supplemental oxygen. He also regained the ability to feed himself small amounts of soft/liquid food with assistance. After the seventh dose, he was able to be weaned off supplemental oxygen. After the eighth and final dose, the patient had improved enough to be transferred to a rehabilitation hospital. A few days later, his tracheotomy and foley catheter were able to be removed. Thereafter, the patient continued his recovery. Within a week he was able to walk 100-feet using a supportive walking device, stand up and sit multiple times, and lift light weights. A week later, he was able to walk 300-feet with a walker and climb stairs. The following week, he returned home and began outpatient therapy. Over the next 3 months, he made a gradual, nearly complete recovery, exhibited no long-term cognitive deficits, and was able to return full-time to his job in the pharmaceutical industry.

Discussion

Our finding that the SARS-CoV-2 spike protein directly binds to the MBL-MASP1/2 complex in human serum suggested the possibility of MASP2 inhibition for the treatment of hospitalized COVID-19 patients. Around the same time that we made this observation, the Omeros anti-MASP2 monoclonal antibody Narsoplimab demonstrated remarkable efficacy in an open label study in six Italian COVID-19 patients requiring mechanical ventilation, all of whom made nearly complete recoveries [12]. The confluence of these two events convinced us to suggest treating a severely ill, COVID-19 patient on a ventilator in the US (in whom all other treatments had failed) with Narsoplimab. To our knowledge this was the first US COVID-19 patient treated with Narsoplimab. Remarkably, following a 4-week course of Narsoplimab, the patient was able to make a nearly complete recovery and returned to work full-time within 3 months of the final dose of Narsoplimab being administered.

Additional recent reports support the rationale of treating severely ill COVID-19 patients with a MASP2 inhibitor. Increasingly, COVID-19 is being recognized as an endothelial cell disease that exhibits characteristics of thrombotic microangiopathy (TMA) [3-5]. It is also being further recognized that the triad of inflammation, complement activation/deposition, and excessive clotting is responsible for the observed COVID-19 pathology, which closely resembles the TMA sometimes seen after hematopoietic stem cell transplant (HSCT-TMA) [18,19]. These three pathways described above intersect at the MBL-MASP1/2 complex, which we now demonstrate is directly engaged by SARS-CoV-2 spike protein. Once MASP2 in this complex is activated, it initiates a positive feedback loop causing further activation of both the clotting and lectin complement pathways. This results in endothelial cell damage in multiple tissues beyond just the lung and systemic excessive clotting reflected by markedly increased levels of circulating D-dimers.

Consistent with these concepts, Narsoplimab is currently being developed for the treatment of HSCT-TMA and IgA nephropathy and has demonstrated compelling efficacy in both diseases [20-22]. While reports have also emerged regarding the use of the C5 inhibitor eculizumab to treat HSCT-TMA and COVID-19 [23-25], C5 inhibition brings with it an increased risk of infection since all three complement pathways converge downstream at C5. This is particularly an issue because the classical complement pathway is a critical component of the adaptive immune system, with patients harboring C5-9 complement deficiencies known to be at risk of Neisseria and related infections. In addition, inhibition of C5 alone does not directly address the excessive clotting accompanying severe SARS-CoV-2 infection. In contrast, inhibition of the upstream target MASP-2 selectively inhibits only the lectin complement pathway (thus leaving the classical complement pathway and the adaptive immune system intact) and also directly suppresses the activation of the clotting cascade caused by MASP2.

Conclusion

The striking improvement in our COVID-19 patient after being administered Narsoplimab suggests that MASP2 inhibition may be a highly desirable treatment option for severely ill COVID-19 patients. It should be noted, however, that our report is a single, anecdotal case report and that and future RCTs will be required to prove the merit of Narsoplimab in the clinical management of COVID-19 infections.

Conflicts of Interest

The authors declare that there are no conflicts of interest among the authors or our institutions.

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