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Umbilical Cord-derived Mesenchymal Stem Cells modulate TNF and soluble TNF Receptor 2 (sTNFR2) in COVID-19 ARDS patients

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Abstract. – OBJECTIVE: We aimed at explaining the mechanism of therapeutic effect of Umbilical Cord Mesenchymal Stem Cells (UC-MSC) in subjects with COVID-19 Acute Respiratory Distress Syndrome (ARDS). Patients with COVID-19 ARDS present with a hyperinflammatory response characterized by high levels of circulating pro-inflammatory mediators, including tumor necrosis factor α and β (TNFα and TNFβ). Inflammatory functions of these TNFs can be inhibited by soluble TNF Receptor 2 (sTNFR2). In patients with COVID-19 ARDS, UC-MSC appear to impart a robust anti-inflammatory effect, and treatment is associated with remarkable clinical improvements. We investigated the levels of TNFα, TNFβ and sTNFR2 in blood plasma samples collected from subjects with COVID-19 ARDS enrolled in our trial of UC-MSC treatment.

PATIENTS AND METHODS: We analyzed plasma samples from subjects with COVID-19 ARDS (n=24) enrolled in a Phase 1/2a randomized controlled trial of UC-MSC treatment. Plasma samples were obtained at Day 0 (baseline, before UC-MSC or control infusion), and Day 6 post infusion. Plasma concentrations of sTNFR2, TNFα, and TNFβ were evaluated using a quantitative multiplex protein array.

RESULTS: Our data indicate that at Day 6 after infusion, UC-MSC recipients develop significantly increased levels of plasma sTNFR2 and significantly decreased levels of TNFα and TNFβ, compared to controls.

CONCLUSIONS: These observations suggest that sTNFR2 plays a mechanistic role in mediating UC-MSC effect on TNFα and TNFβ plasma levels, determining a decrease in inflammation in COVID-19 ARDS.

Key Words:
Mesenchymal Stem Cells (MSC), Tumor Necrosis Factor (TNF), soluble TNF Receptor 2 (sTNFR2), COVID-19, Acute Respiratory Distress Syndrome (ARDS), Anti-inflammatory effect, Hyperinflammatory response, Immunomodulation, Umbilical Cord, UC-MSC.

Introduction

SARS-CoV-2 infection can cause severe COVID-19, which is characterized by exaggerated immune and inflammatory responses, cytokine release syndrome (*a.k.a.*, cytokine storm), and Acute Respiratory Distress Syndrome (ARDS)[1]. Therapeutic interventions shall target the hyperinflammatory state in these patients. Mesenchymal Stem Cells (MSC) have been proposed as a therapeutic modality due to their strong immunomodulatory, anti-inflammatory, and reparative properties[2,3]. MSC have been widely used in the allogeneic setting, which has advantages in the context of emergency response. These cells have potential for counteracting the excessive immune response and attenuate lung injury in patients with severe COVID-19 (reviewed in[4]).

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We recently conducted a double-blind Phase 1/2a randomized controlled trial of Umbilical Cord MSC (UC-MSC) treatment in 24 patients with COVID-19 ARDS[5]. We observed that UC-MSC treatment was associated with remarkable clinical benefits, including significant improvements in survival (91% *vs.* 42% at 1 month, p=0.015), serious adverse events-free survival (p=0.008), and time to recovery (p=0.03), when compared to control group[5]. The mechanism of effect of UC-MSC in COVID-19 ARDS needs to be elucidated. UC-MSC appear to modulate inflammation in patients with COVID-19 ARDS. Tumor Necrosis Factors (TNF) α and β are master regulators of inflammation. Soluble TNFR2, on the other hand, is capable of binding TNF, neutralizing TNF-induced cytotoxicity and immune-reactivity, resulting in inhibition of inflammatory responses[6]. To help explain the mechanism of therapeutic effect of UC-MSC in subjects with COVID-19 ARDS, we evaluated plasma concentrations of TNFα, TNFβ, and sTNFR2, in subjects enrolled in our clinical trial.

Patients and Methods

Plasma concentrations of soluble tumor necrosis factor receptor 2 (sTNFR2), tumor necrosis factor alpha (TNFα), and tumor necrosis factor beta (TNFβ) were evaluated in subjects with COVID-19 acute respiratory distress syndrome (ARDS) enrolled in our Phase 1/2a clinical trial (n=24). Blood samples were obtained from subjects at day 0 (before infusion) and day 6 (3 days after second infusion). Briefly, whole blood was collected into EDTA-treated tubes, transferred on ice, and processed for plasma separation within 2 hours. Whole blood was centrifuged at 2,000 g for 15 min at 4°C, plasma was collected and stored at –80°C until processing. A quantitative multiplex protein array (RayBio® Q-Series, RayBiotech, GA, USA) was utilized to determine the plasma levels of sTNFR2, TNFα, and TNFβ (pg/ml) - processing all samples at the same time, following manufacturer's instructions. The fluorescent signals were visualized via a Cy3 wavelength laser scanner and converted to concentrations using the standard curve generated per array.

Statistical Analysis

Statistical analysis was performed using two sample T-tests and nonparametric Wilcoxon two-sample tests. Signed rank tests were used for paired comparisons examining changes between timepoints within group. All tests were two-sided, with statistical significance established with $p<0.05$. Data are presented with means and standard errors of the mean.

Results

Patients in UC-MSC and control groups showed no significant difference in protein levels at baseline (Day 0, Figure 1). In control group, levels of plasma sTNFR2, TNFα, and TNFβ were not significantly different between days 0 and 6. In UC-MSC treatment group, TNFα and TNFβ levels decreased significantly (p=0.005 and p=0.002, respectively) from day 0 to day 6. Comparisons between groups on day 6 demonstrated that UC-MSC treatment group had significantly higher levels of sTNFR2 (26.609±846 pg/ml *vs.* 23.111±760 pg/ml, p=0.021), and significantly lower levels of TNFα (319±40 *vs.* 950±226 pg/ml, p=0.048) and TNFβ (810±126 *vs.* 2.944±735 pg/ml, p=0.032) compared to control group (Day 6, Figure 1).

Discussion

COVID-19 is continuing to spread worldwide. Due to the high mortality in severe COVID-19, there is urgent need for therapies that attenuate excessive inflammation and accelerate recovery. In our Phase 1/2a randomized controlled trial of Umbilical Cord MSC (UC-MSC) in COVID-19 ARDS, we observed remarkable clinical benefits of UC-MSC treatment with significant improvements in survival, serious adverse events-free survival, and time to recovery compared to control group[5].

Herein, we elucidate a potential key mechanism of UC-MSC effect in COVID-19 ARDS patients. UC-MSC treatment results in an increase in sTNFR2, a soluble receptor that binds to TNFα and TNFβ, and a decrease in the levels of these two cytokines. Notably, studies showed that higher sTNFR2 levels lead to decreased T cell activation and increased production of regulatory T cells (T regs)[7]. Specifically, sTNFR2 expression in MSC is correlated to an increased ability to induce $Foxp3^+$ T regs[8].

sTNFR2 has been used as the base for potent pharmacological agents that inhibit TNF function, such as Etanercept. These agents have been utilized extensively to treat chronic inflammatory

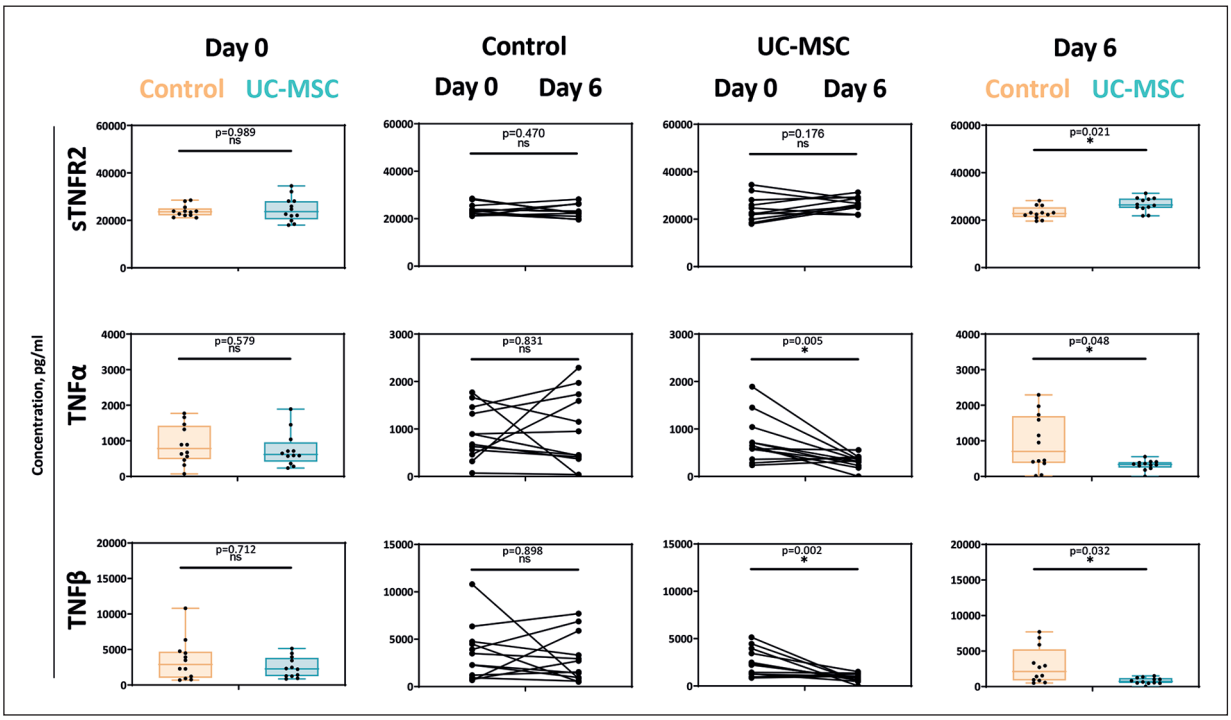

Figure 1. Plasma concentrations of soluble tumor necrosis factor receptor 2 (sTNFR2), tumor necrosis factor alpha (TNFα), and tumor necrosis factor beta (TNFβ) in subjects with COVID-19 acute respiratory distress syndrome (ARDS) (n=24). Patients in UC-MSC and control groups showed no significant difference in protein levels at baseline. In control group, levels of sTNFR2, TNFα, and TNFβ were not significantly different between days 0 and 6. In UC-MSC group, TNFα and TNFβ levels decreased significantly (p=0.005 and p=0.002, respectively) from day 0 to day 6. Comparisons between groups on day 6 demonstrated that UC-MSC treatment group had significantly higher levels of sTNFR2 (26.609±846 pg/ml *vs*. 23.111±760 pg/ml, p=0.021), and significantly lower levels of TNFα (319±40 *vs*. 950±226 pg/ml, p=0.048) and TNFβ (810±126 *vs*. 2.944±735 pg/ml, p=0.032). Data are presented as box and whiskers plots indicating the median values and min to max values, and as scatter plots with lines indicating individual values.

and autoimmune diseases[9], and could also be beneficial for COVID-19 ARDS[10]. TNF blockade is clinically effective in inflammatory settings as it results in rapid reduction of the levels of interleukin-1 (IL-1), IL-6, adhesion molecules and vascular endothelial growth factor (VEGF), ultimately inhibiting leukocytes trafficking and capillary permeability in inflamed tissues (reviewed in [10]). Therefore, the soluble receptor sTNFR2 may be a central mediator of the anti-inflammatory effect of UC-MSC treatment. These observations are relevant in the development of therapeutics for COVID-19 and inflammatory disorders.

Conclusions

Our findings propose that sTNFR2 plays a key mechanistic role in mediating UC-MSC effect on TNFα and TNFβ plasma levels to decrease hyperinflammation in COVID-19 ARDS.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

GL and CR conceived the study. CR is the principal investigator. GL, DC, SMC, PR and CR designed the trial. DK, DC, LDS and GL contributed to analyses and data entry. DK and SMC conducted the statistical analyses. EL and CL were responsible for UC-MSC manufacturing. All authors contributed to the preparation of the report. All authors critically reviewed and approved the final version.

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