Development of a Novel Interleukin-27 (IL-27) Targeted Gene Therapy for Therapeutic Applications

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Summer Research Opportunities Program (SROP)
OFFICE OF GRADUATE DIVERSITY INITIATIVES

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INTRODUCTION

SARS-CoV-2 relies on the angiotensin-converting enzyme 2 (ACE2) transmembrane peptidase as its cellular receptor and is responsible for **7,000,000 global deaths** since 2019[1]. Current therapeutics (e.g., plasmapheresis, hydroxychloroquine, etc.) prove ineffective in many patient populations and support the need for more targeted therapies[2].

Interleukin-27 (IL-27) is a pleiotropic cytokine with anti-inflammatory and anti-viral properties[3]. We designed a novel plasmid encoding IL-27 with an ACE2-targeting peptide (IL-27^{ACE2pep}) and proposed an IL-27 delivery via adipose stromal mesenchymal cells (ASC) to models of lung inflammation, exploring *in vitro* potential of ASC in delivering therapeutics to target cells. Elucidating the effects of IL-27^{ACE2pep} hASC CM on A549-ACE2 establishes a foundation for developing life-saving IL-27 targeted therapies for COVID-19 and a multitude of other conditions, such as ACE2-overexpressing tumors and autoimmune conditions.

HYPOTHESIS

We hypothesize that human ASC conditioned media (hASC CM) containing IL-27^{ACE2pep} will **reduce** both **ACE2 expression** and **viral entry** in A549-ACE2 cells, inhibiting inflammation.

RATIONALE

Our *in silico* analysis demonstrated the remarkable ability for IL-27 to induce antiviral genes and to restore cytokine balance, positioning this as a potentially life-saving translational therapy. When previously tested on HEK293-ACE2, IL-27^{ACE2pep} hASC CM caused a STAT1-mediated upregulation of ACE2, increasing SARS-CoV-2-Spike pseudotyped lentivirus entry. Thus, we sought to determine whether A549-ACE2, a lung epithelial cell line, would elicit the same response.

The applications of this therapy extend to a plethora of immune reactions associated with cytokine release syndrome, including autoimmune diseases, viral infections, and immunotherapy treatments for cancer (such as CAR T-cell therapy). Additionally, this therapy can be applied as anti-cancer agents for tumors overexpressing ACE2, such as papillary renal cell carcinoma (94% overexpress ACE2)[4]. Therefore, the preclinical experimentation on models of lung inflammation are promising for a wide variety of therapeutic applications and reinforce the importance of this research.

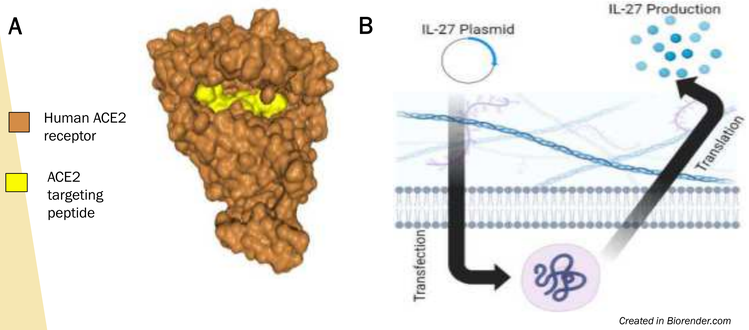


Fig 1. Overview of Novel IL-27^{ACE2pep} Gene Delivery

(A) Predicted binding of the ACE2-targeting peptide to Human ACE2 receptor using HPEPDOCK.

(B) Transfection of the IL-27 plasmid initiates translation and consequential production of IL-27. Human adipose stromal/stem cells (hASC) were used to produce IL-27, and the conditioned media was collected and stored for future experiments.

METHODOLOGY AND RESULTS

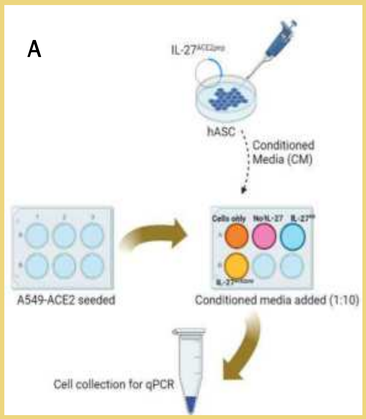
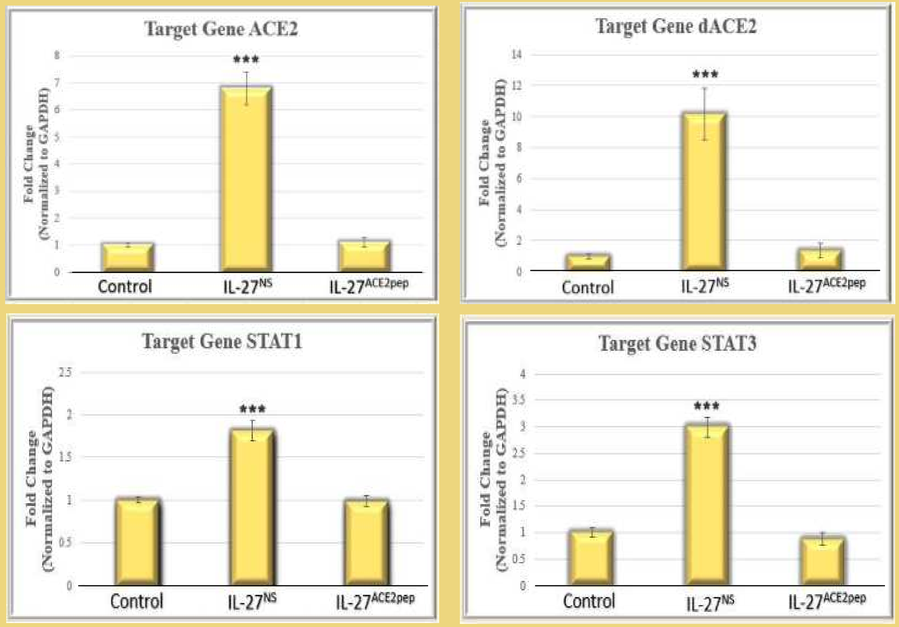


Fig 2. Determining gene expression in A549-ACE2 cells following treatment of IL-27 hASC CM

(A) Methodology overview to determine gene expression in A549-ACE2 cells following treatment of IL-27 hASC CM. 150,000 A549-ACE2 cells/well were seeded in a six-well plate. The cells were then treated with conditioned media with the following treatments: cells only, empty vector (No IL-27), non-targeting IL-27 (IL-27^{NS}), and ACE2 targeting IL-27 (IL-27^{ACE2}). The cells were collected 48 hours after treatment and analyzed via qPCR.

(B) Gene expression fold change in A549-ACE2 cells treated with IL-27^{NS} and IL-27^{ACE2}. Target genes analyzed were ACE2, dACE2, STAT1, and STAT3, normalized to GAPDH. Data is represented as mean ± S.D. (***p<.001).

B



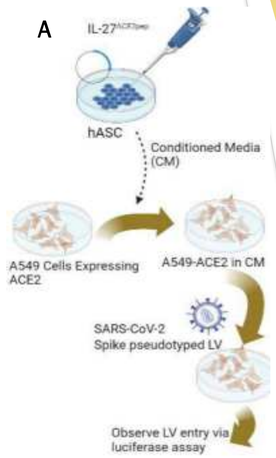


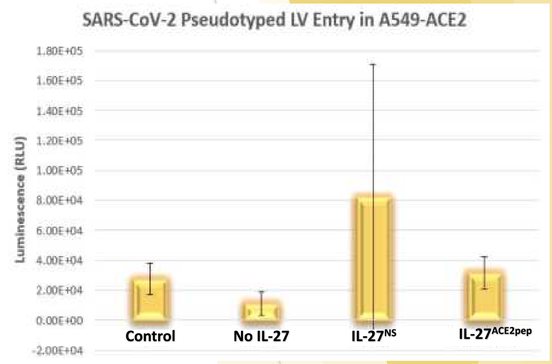
Fig 3. Determining SARS-CoV-2-Spike pseudotyped lentivirus entry in A549-ACE2 following treatment of IL-27 hASC CM

(A) Methodology overview to determine SARS-CoV-2-Spike pseudotyped lentivirus entry in A549-ACE2. Cells were seeded in 96-well plate at 5,000 cells/well. The cells were then treated with/without conditioned media (same as Fig 3) for 4 hours before media is replaced, and pseudotyped LV was added. Luciferase assay was conducted at 48h post LV transduction.

(B) SARS-CoV-2-Spike pseudotyped LV entry in A549-ACE2 measured in luminescence (RLU). SARS-CoV-2 pseudotyped LV expresses synthetic firefly luciferase (Luc2). Data is represented as mean ± S.D. and paired t-test was used to determine significance.

(C) Representative images of A549-ACE2 taken at 48 hours post LV transduction. SARS-CoV-2-Spike pseudotyped LV expresses green fluorescence protein (ZsGreen1). Images were taken using an inverted fluorescent microscope (Olympus IX71) at 10X magnification.

B



C

DISCUSSION & FUTURE DIRECTIONS

Our *in vitro* analysis demonstrated **a reduced trend of LV entry** following treatment **with IL-27^{ACE2pep}** compared to IL-27^{NS}. This is likely due to the **differences in ACE2 expression**, in which **non-specific IL-27** (IL-27^{NS}) hASC CM significantly **upregulated ACE2 expression**, whereas **IL-27^{ACE2pep} CM did not**. Analyzed together, the results suggest existing differences in responses between HEK293-ACE2 and A549-ACE2 cells to our therapy and show promise in mitigating the frequently life-threatening effects of COVID-19 and other conditions. Additionally, the demonstrated ability for IL-27^{ACE2pep} to downregulate ACE2 expression in A549-ACE2 cells is significant for the field of oncology and represents substantial potential in translational therapy for tumors overexpressing ACE2.

Future directions include further **optimization** of the protocol for SARS-CoV-2-Spike pseudotyped LV entry assay in order to more accurately determine viral entry and then analysis of **STAT1 activity** following treatment of A549-ACE2 cells.

AUTHOR CONTRIBUTION STATEMENT

JD: Design of the poster and graphical illustrations in Figures 1B, 2A, 3A; contributed and generated data in Figures 2B and 3B
GM: Design of the experiments; generated the graphical illustration in Figure 1A and data in Figure 3C
WS: Design and cloning of the IL-27 plasmids
MLF: Oversaw the project design and execution; contributed funding

ACKNOWLEDGEMENTS

A549-ACE2 cell line and SARS-CoV-2 pseudotyped lentivirus were obtained from BEI Resources (NR-53522 and NR-53816). This project was supported by a pilot grant from the Indiana Clinical and Translational Sciences Institute and was funded by Award Number UL1TR002529 from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences Award. This project was also supported by The Summer Research Opportunities Program. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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