Tocilizumab is effective in reducing inflammation in Type 2 diabetes-COVID-19 organ-on-a-chip model

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ABSTRACT

The COVID-19 pandemic has contributed to more than 5 million deaths worldwide in the last two years. Co-morbid conditions such as Type2 Diabetes (T2D), hypertension, obesity, chronic kidney disease have been associated with increased mortality with COVID-19. In a large meta-analysis, the relative risk of mortality was 1.54 for patients with T2D and COVID-19. Thus, there is an imperative need to develop a platform for rapid and reliable drug screening/selection against COVID-19 related morbidity/mortality in T2D patients. With limited translatability of in vitro and small animal models to humans, human organ-on-a-chip models are an attractive platform to model in vivo disease conditions and test potential therapeutics. We seeded T2D or non-diabetic patient-derived macrophage and human liver sinusoidal endothelial cells along with normal hepatocytes and stellate cells in the liver-on-a-chip (LAMPS - Liver Acinus Micro Physiological System) developed by our group, perfused with media mimicking normal fasting or late metabolic syndrome (LMS - high levels of glucose, fatty acids, insulin, glucagon, cytokines) states. We transduced both macrophage and endothelial cells to overexpress the SARS-CoV2-S (spike) protein and compared it with a control lentivirus transduction. We found that T2D cells overexpressing S-protein in LMS media (T2D-COVID-19-LAMPS) displayed an increased secretion of inflammatory cytokines compared to the non-diabetic-COVID-19-LAMPS. We then tested the effect of Tocilizumab (IL6-receptor antagonist) in T2D-COVID-19-LAMPS. Compared to vehicle control, Tocilizumab significantly decreased the S-protein induced inflammatory cytokine secretion in T2D LAMPS but not in non-diabetic-LAMPS, indicating its higher efficacy in severe disease states only. This is consistent with what was observed in large clinical trials providing confirmatory evidence that the T2D-COVID-19-LAMPS and non-diabetic-COVID-19-LAMPS serve as a relevant in vitro model system to replicate human in vivo pathophysiology of COVID and for screening potential therapeutics.

BACKGROUND

- T2D is 2.5 times more prevalent among fatal cases of COVID-19 as compared to total.
- Organ-on-a-chip are reliable, rapid and efficient model for drug testing.

AIM & OBJECTIVES

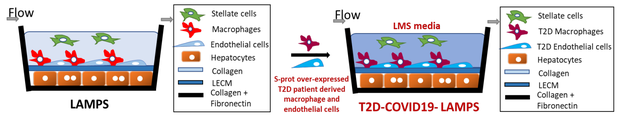
To develop a organ-on-a-chip platform for testing drugs for T2D-COVID-19 patients

- To develop and validate liver organ-on-a-chip for mimicking T2D-COVID-19 model
- Test potential therapeutics in the model

EXPERIMENTAL DESIGN

LAMPS: Liver Acinus Micro Physiological System

1A



1B

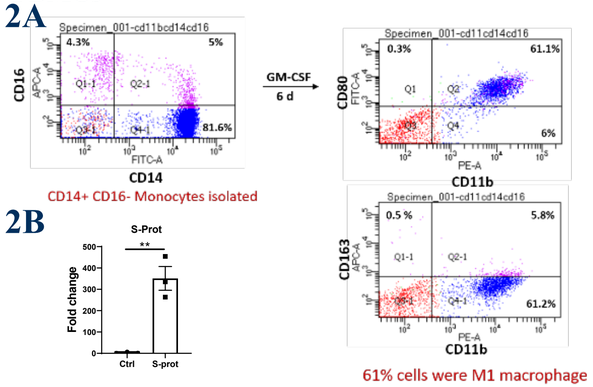
Component	NF (Normal Fasting)	LMS (Late Metabolic Syndrome; NAFLD, T2D)
Glucose	5.5 mM	20 mM
Insulin	10 pM	10 nM
Glucagon	100 pM	10 pM
Oleic acid	-	200 uM
Palmitic acid	-	100 uM
LPS	-	1 ug/mL
TGF-β	-	10 ng/mL
Glutamine (glutaMAX)	2mM	2mM

Figure 1A. Schematic of LAMPS, it consists of sequential addition of different human liver cell types namely-hepatocytes, macrophages, endothelial and stellate cells on collagen-fibronectin matrix. To convert it to T2D-COVID-19-LAMPS, T2D patient derived macrophage and endothelial cells were over-expressed with SARS-CoV2-Spike protein and cultured in LMS (Late metabolic Syndrome) media. Non-diabetic cells, empty backbone vector and NF (Normal Fasting) media were used as control. **B.** Media composition of NF and LMS media that were used to mimic non-diabetic and T2D condition in LAMPS, respectively. Briefly, LMS contains higher concentration of glucose, insulin, glucagon, fatty acid and inflammatory cytokines .

RESULTS

I- Differentiation of primary macrophages from PBMCs

2A

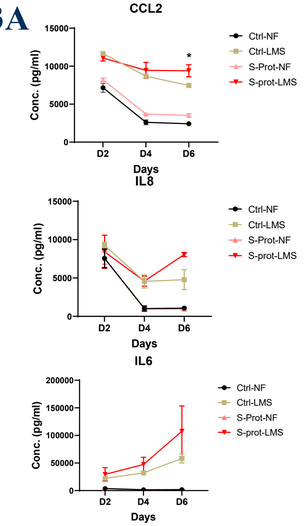


2B

Figure 2. Monocytes obtained from PBMCs (peripheral blood mononuclear cells) were differentiated into macrophages in vitro . **A.** Classical monocytes (CD14+ CD16-) isolated from PBMC were found to be 81% pure and were further differentiated into M1 macrophages by culturing in presence of GM-CSF for 6 days. Percentage of differentiation were determined by flow cytometry by staining for M1 (CD80+) and M2 (CD163+) markers of macrophages (CD11b). **B.** Lentivirus based SARS-CoV-2-S- protein was transduced in these macrophages and was validated at RNA level using qRT-PCR at 72h post transduction.

II- Inflammatory cytokines were increased in LAMPS with LMS media

3A



3B

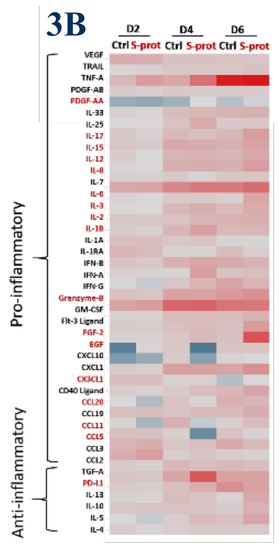
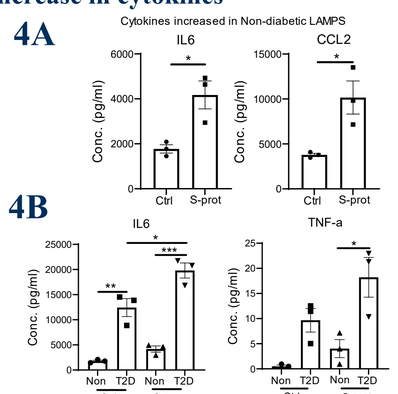


Figure 3. SARS-CoV-2-S-protein (S-prot) was over-expressed in macrophages and cultured in NF and LMS media corresponding non-diabetic and T2D condition in LAMPS respectively. The cytokines secreted are represented as **A.** line graph and **B.** heat map, indicating slight increase with S-protein in LMS media.

III- Use of T2D patient derived cells and S-protein over-expression in LSEC further amplified the increase in cytokines

4A



4B

C

4C

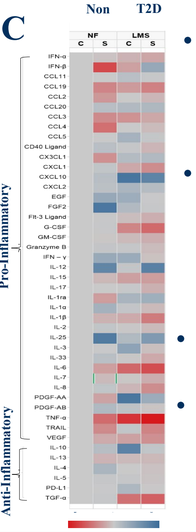
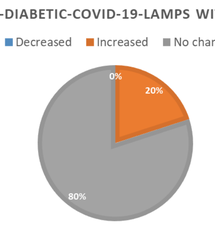


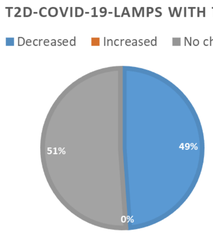
Figure 4. SARS-CoV-2-S-protein (S-prot) was over-expressed in both macrophages and LSEC of non-diabetic and T2D patient derived cells, hence the name non-diabetic-COVID-19 and T2D-COVID-19 LAMPS. **A**. Among many, IL6 and CCL2 are shown here that were increased in non-diabetic-COVID-19-LAMPS by S-prot, **B**. Cytokines (IL6 and TNF-a) were amplified in T2D-COVID19-LAMPS. **C**. Heat-map indicating overall log2 fold change in cytokines w.r.t non-diabetic non-COVID-19 LAMPS

IV- Tocilizumab decreased the inflammatory cytokines in T2D-COVID-19 chip but not in non-diabetic-COVID-19 chip

5A



5B



5C

	S-T2D-VC	S-T2D-Toz
IFN-α	1	0.55253
IFN-β	1	1.210545
CCL11	1	0.831798
CCL19	1	0.7404
CCL2	1	0.479204
CCL20	1	0.673308
CCL3	1	0.730774
CCL4	1	0.854759
CCL5	1	0.532981
CD40 Ligand	1	0.604634
CX3CL1	1	0.729099
CXCL1	1	0.643085
CXCL10	1	1.251961
CXCL2	1	0.509085
EGF	1	0.635022
FGF2	1	0.444734
Flt-3 Ligand	1	0.742727
G-CSF	1	0.394508
GM-CSF	1	0.597317
Granzyme B	1	0.624086
IFN-γ	1	1.152913
IL-12	1	1.22262
IL-15	1	0.698672
IL-17	1	0.981538
IL-1ra	1	0.628796
IL-1α	1	0.743595
IL-1β	1	0.719004
IL-2	1	0.753205
IL-25	1	0.420892
IL-3	1	0.628622
IL-33	1	0.623163
IL-6	1	0.501934
IL-7	1	0.847345
IL-8	1	0.767675
PDGF-AA	1	1.389291
PDGF-AB	1	0.896309
TNF-α	1	0.40701
TRAIL	1	0.902362
VEGF	1	0.588349
IL-10	1	1.126539
IL-13	1	0.876525
IL-4	1	0.232939
IL-5	1	0.899064
PD-L1	1	0.829385
TGF-α	1	0.673562

Pro-Inflammatory / Anti-Inflammatory

Figure 5. Both Non-diabetic-COVID-19 and T2D-COVID-19-LAMPS were cultured in presence of Tocilizumab (100 ug/ml) for 36h. Pie graph representing cytokines altered by Toz in **A.** non-diabetic-COVID-19-LAMPS and **B**. T2D-COVID-19-LAMPS is shown. **C**. Heat-map showing overall changes in cytokines by Toz in T2D-COVID-19-LAMPS.

CONCLUSION

- Tocilizumab decreased the S-protein induced inflammatory cytokine secretion in T2D-COVID-19-LAMPS but not in non-diabetic-COVID-19-LAMPS
- Validated T2D-COVID-19-LAMPS as a relevant model to replicate human in vivo pathophysiology of COVID and for screening potential therapeutics

FUTURE DIRECTIONS

- Determine the underlying mechanism of severity of COVID-19 infection in T2D patients
- Test efficacy of other new drugs

ACKNOWLEDGEMENT

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