040

The Transcriptome Landscape of 3D-cultured Placental Trophoblasts Reveals Activation of TLR2 and TLR3/7 in Response to *Trypanosoma cruzi*

Erica Silberstein[1], Charles C. Chung[2] and Alain Debrabant[1]
[1] OBRR/DETTD/LEP; [2] CBER/HIVE

Introduction

Chagas Disease and Congenital Transmission

- Chagas disease (CD) is caused by the bloodborne protozoan parasite *Trypanosoma cruzi (T. cruzi).*
- CD is transmitted by triatomine bugs, from **mother-to-baby** and by blood transfusion and organ transplantation.
- Congenital infections *a*ccount for over 20% of new cases worldwide.
- The risk of vertical transmission is 1-5 %.
- 40,000 infected women of childbearing age live in the United States, where an estimated 63-315 babies acquire *T. cruzi* infection from their mothers per year.
- The placental syncytiotrophoblasts (SYNs) form an effective physical and immunological barrier against pathogens.
- *T. cruzi* parasites circulating in maternal blood can be transmitted to the fetus through the SYNs.

Previous Findings

- We have found that 3D-grown cultures of SYNs exhibit morphological and secretory characteristics comparable to placental trophoblasts (form syncytia and express hormones) and are highly resistant to *T. cruzi* infection. *Silberstein et al. Fron. Microbiol.2021; doi:10.3389/fmicb.2021.626370.*

The Human Placenta

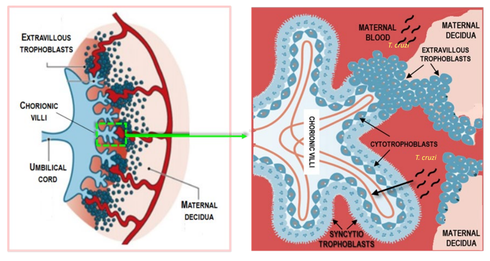


Figure 1. Structure of the human placenta. Adapted from "Immune responses at the maternal-fetal interface"; Sci Immunol. 2019; doi: 10.1126/sciimmunol.aat6114.

Objectives

- Identify the defense mechanisms that restrict *T. cruzi* placental infection using a three-dimensional (3D) cell culture system of human syncytiotrophoblasts (SYNs).
- Explore the role of Toll-like receptors in the control of *T. cruzi* infection of the placenta.

Methodology

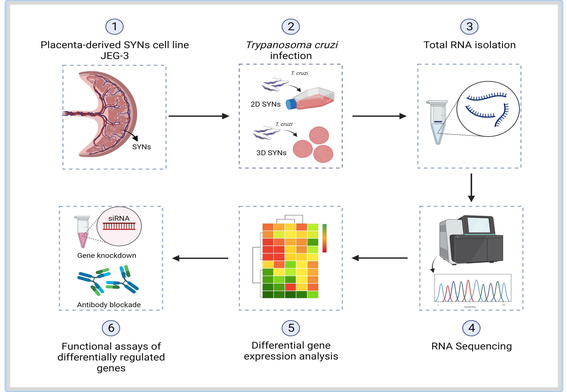
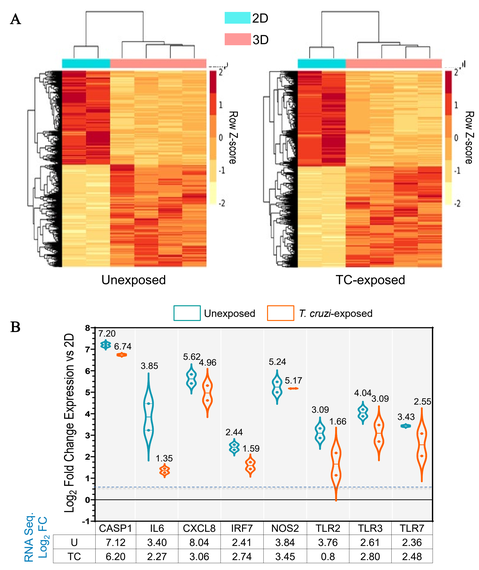


Figure 2. Experimental workflow for the whole transcriptome analysis of 2D and 3D-grown SYNs cultures unexposed or exposed to *T. cruzi.*

Results

Alterations in global gene expression profiles of 3D and 2D-grown SYNs



	CASP1	IL6	CXCL8	IRF7	NOS2	TLR2	TLR3	TLR7
U	7.12	3.40	8.04	2.41	3.84	3.76	2.61	2.36
TC	6.20	2.27	3.06	2.74	3.45	0.8	2.80	2.48

Figure 3. (A) Hierarchical cluster analysis of normalized read counts of differentially expressed genes between 3D and 2D SYNs unexposed or exposed to *T. cruzi.* (B) Validation of RNA-Seq data by qRT-PCR. mRNA quantification of selected genes carried out by qRT-PCR in unexposed or *T. cruzi*- exposed 3D SYNs.

Results

Pathway analysis reveals activation of processes associated with cellular immune defenses, pathogen-influenced signaling and cellular stress/ injury in 3D-cultured SYNs

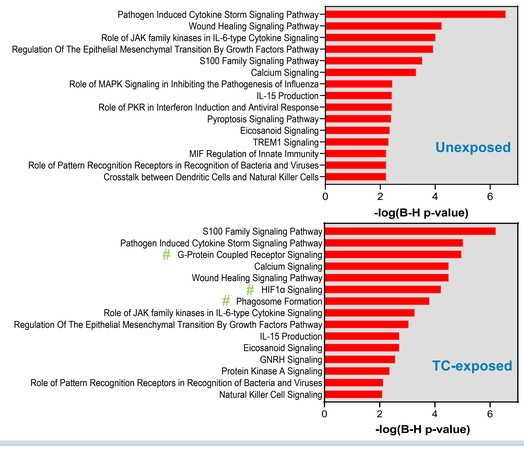


Figure 4. Top significant canonical pathways identified by IPA in 3D SYNs compared to 2D SYNs, in unexposed and *T. cruzi*-exposed cultures. The red-colored bars represent the -log (B-H *p*-value) calculated for each pathway. Only pathways with z-score ≥ 2 and B-H *p*-value ≥ 0.05 were considered significant.

Toll-like receptors and cytokines are common genes in the top activated canonical pathways

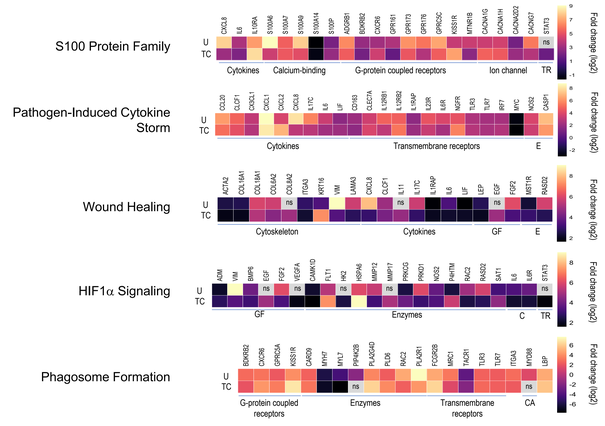


Figure 5. Heatmaps of top differentially expressed genes. Only genes with $\log_2$ fold change ≥ ± 1.5 and *p*-value ≥ 0.05 were considered significant. ns: no significant $\log_2$ fold change or B-H *p*-values. U: unexposed; TC: *T. cruzi*-exposed. CA: cytosolic adapter protein; C: cytokines and cytokine receptors; E: enzymes; GF: growth factors; TR: transcription regulators.

Inhibition of TLRs significantly decreases expression of cytokines and interferons promoting *T. cruzi* growth

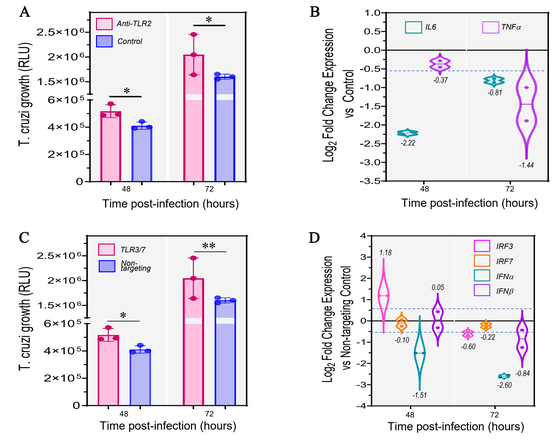
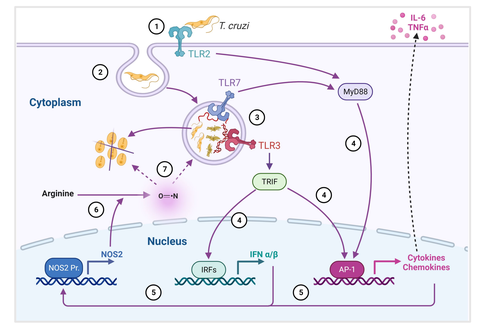


Figure 6. 2D SYNs were treated with anti-hTLR2 (TLR2 blockade) or transfected with TLR3/ TLR7 siRNA mix, followed by *T. cruzi* infection. **(A)** *T. cruzi* growth over time after TLR2 blockade. **(B)** qRT-PCR mRNA quantification after TLR2 blockade. **(C)** *T. cruzi* growth over time after TLR3/ TLR7 siRNA transfection. **(D)** qRT-PCR mRNA quantification after TLR3/ TLR7 siRNA transfection. *: $p \leq 0.05$; **: $p \leq 0.01$.

Model of immune response mechanisms against *T. cruzi* mediated by Toll-like receptors in 3D-grown SYNs



Conclusions

SYNs resist infection by constitutively expressing pro-inflammatory molecules and modulating multiple defense mechanisms that interfere with the parasite's intracellular life cycle, contributing to parasite killing and infection control