Evaluation of Macrophage mRNA Expression after Conditioned Cancer Media Treatments

Warsan Artan[1], Joi Weeks[1], Oscar Echeagaray[1], Charles Vallez[1], Dr. Natalie Gude[1], and Dr. Mark Sussman[1]
[1]Biology Department, San Diego State University, San Diego, CA 92182, USA

San Diego State University • UC San Diego Moores Cancer Center
PARTNERSHIP SCHOLARS
outreach | education | research

SAN DIEGO STATE UNIVERSITY

UC San Diego Health
MOORES CANCER CENTER

BACKGROUND

The immune system plays an important role in defending the body of the host and protecting against foreign invaders such as cancer. This system is comprised of both innate and adaptive immunity. Antigen presenting cells (APCs) such as dendritic cells (DCs) and macrophages (Macs) are mostly members of the innate immune system. APCs engulf antigens in a process called phagocytosis. Antigens are processed, bound to the major histocompatibility complex/class II (MHC II) within the endosome of APCs, and transported to the cell surface as part of the APC maturation process. Once APCs mature, antigen presentation occurs and cytokine release is observed.

In this experiment, we exposed Macs (RAW cells) to media preconditioned by colon cancer cells (CT26) treated by heat, mechanical disruption, and starvation to release cancer antigens and determine if these treatment strategies induce inflammatory cytokine synthesis on maturing Macs.

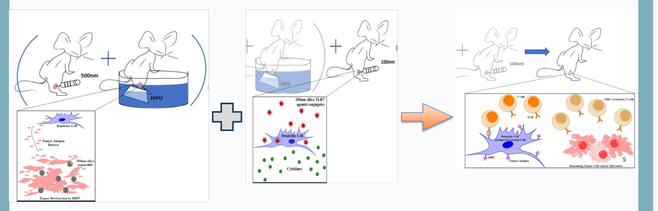
The the purpose of this study is to contribute to Project 1 of the U54 collaboration. Parent Project 1 uses a combination of 500nm hollow nanoshells coupled with non-thermal High Intensity Focused Ultrasound (HIFU) and 100nm nonparticle/nanoshells coated with TRL7 agonist (1v209) to enhance Macs maturation and concomitant antigen presentation.

We hypothesize that the heat shock treatment will cause more cell death, leading to increased antigen release from the CT26 cells, inducing higher expression of cytokine mRNAs, indicative of Macs maturation.

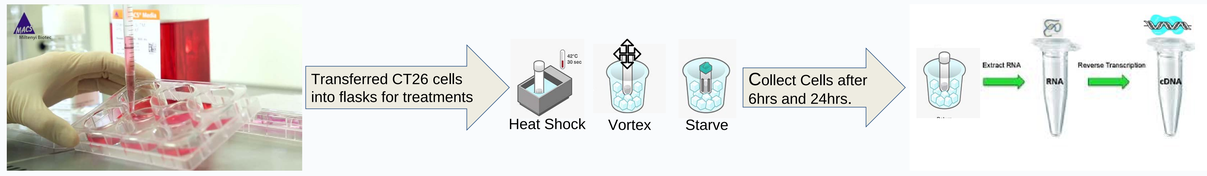
SPECIFIC AIMS

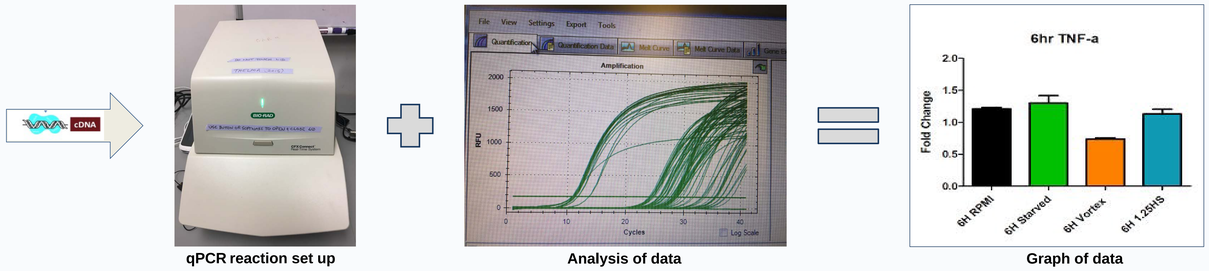
To detect the effects each treatment has on Macs maturation, the aims of this study are:

1. To determine cell death levels of CT26 cells after heat shock, mechanical disruption, and starvation at 6 hours and 24 hours.
2. To measure and analyze cytokine transcript levels from RAW cells treated with CT26 media from aim 1.
3. To establish a baseline cytokine evaluation that will later be used to compare TLR7 conjugated nanoshell treatment to free TLR7 in cancer antigen matured Macs.



METHODS





qPCR reaction set up | Analysis of data | Graph of data

RAW cells were plated into 6-well dishes, and CT26 cells passaged into T25 flasks. The next day, CT26 cells were subjected to environmental stresses (starvation, vortexing, and heat shock). Conditioned media from treated and control CT26 cells was applied to RAW cells. Cells were collected after 6 hours and 24 hours, RNA was purified, and cDNA synthesized for use in RT-qPCR reactions to quantify cytokine transcription levels by RAW cells in response treatment with CT26 conditioned media.

RESULTS

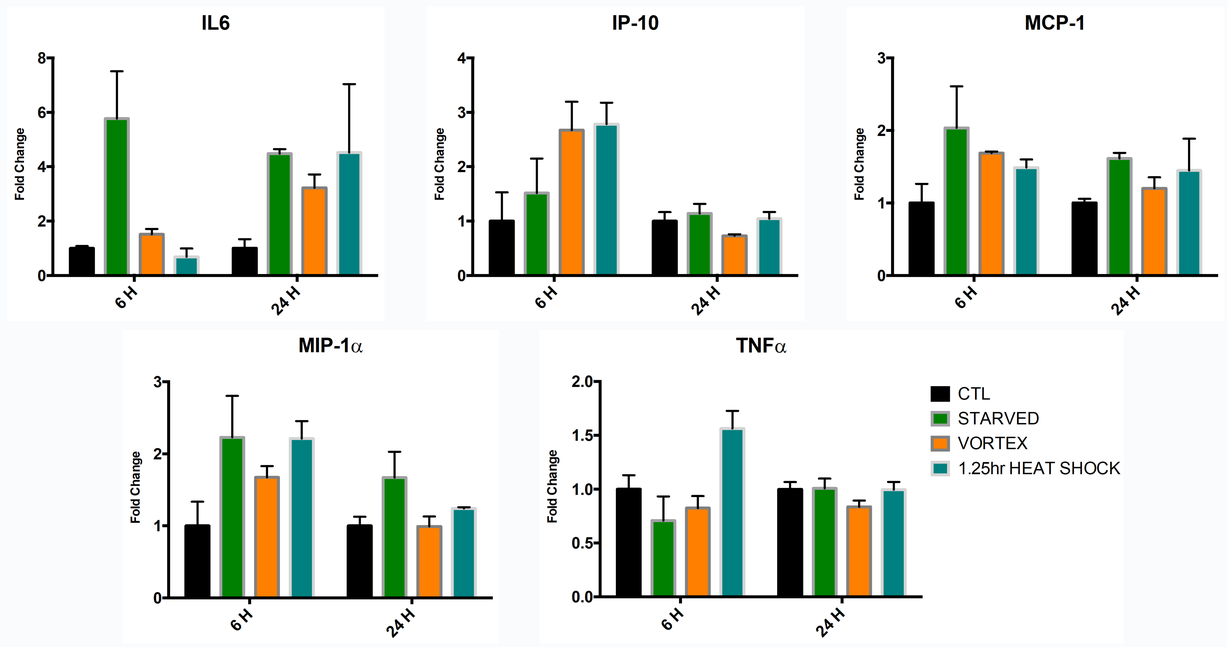


Figure 1: CT26 conditioned media stimulates cytokine expression in RAW cells.
RAW cells were treated with stressed-induced CT26 media and RNA collected after 6 hours and 24 hours. Transcript levels for IL-6, IP-10, MCP-1, MIP-1α, and TNF-α were measured by qPCR and represented as fold change over control using the ΔCT calculation. (starvation=green, mechanical distruption=orange, and 1.24 hr HS in blue).

CONCLUSIONS

Although our hypothesis was not proven, there are noticable changes in the cytokine transcript levels:

- **Cell death:** Heat shock treatment induced highest cell death in CT26 cells (data not shown)
- **IL-6:** All treatments stimulate transcription of IL-6 over by 24 hr.
- **IP-10:** CT26 treated supernatants induce a slight increase of IP-10 transcription in RAW cells at 6 hr but not at 24 hr.
- **MCP-1:** MCP-1 expression is not significantly induced by any treatments at 6 hr or 24 hr.
- **MIP-1α:** MIP-1α expression is not significantly induced by any treatments at 6 hr or 24 hr.
- **TNF-α:** TNF-α expression is not significantly induced by any treatments at 6 hr or 24 hr.

It might be beneficial to test target cytokine mRNA expression after two hours, twelve hours, eighteen hours, and thirty-four hours in order to established a more detailed time course. More time points would demonstrate the type of effects the CT26 media has on macrophage maturation as measured by cytokine transcript levels.
The broader significance of this work is to contribute to the Parent Project 1. Once we know which treatment causes most cell death and antigen release and leads to highest cytokine transcript levels by the antigen-presenting cells (APCs), in this case macrophages, then we can have a baseline for the parent project.

REFERENCES

1.Chen DS & Mellman I (2013) Oncology meets immunology: the cancer-immunity cycle. *Immunity* 39(1):1-10.
2. Gardner, A. & Ruffell, B. Dendritic Cells and Cancer Immunity. Trends in Immunology 37, 855–865 (2016).
3. Janeway CA Jr, Travers P, Walport M, et al. 2001. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science.
4. Shinchi, H. et al. Enhancement of the Immunostimulatory Activity of a TLR7 Ligand by Conjugation to Polysaccharides. Bioconjugate Chemistry 26, 1713–1723 (2015).

ACKNOWLEDGEMENTS

I would like to thank Dr. Crowe, Dr. Bernstein, and Jennifer Suggs for giving me this opportunity. I would also like the to thank Dr. Sussman and Dr. Natalie Gude for giving me the opportunity to work in their lab and Dr. Natalie for constantly checking up on my progress. Last but not least, I would like to extend my greatest gratitude to Joi Weeks for being just an amazing mentor and for always challenging me. I would like to show my deepest appreciation for Oscar Echeagaray and Nick Vallez for their tremendous assistance during this project.

Research reported in this poster was supported by the National Cancer Institute of the National Institutes of Health under award numbers: U54CA132384 & U54CA132379