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 treatments induce inflammatory cytokine synthesis on maturing Macs.

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.

**METHODS**

**RESULTS**

**CONCLUSIONS**

Though our hypothesis was not proven, there are noticeable 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 establish a more detailed time course. More 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**


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