The effect of microgravity on cytokine production in response to antigens

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Over the past few years, there has been increasing interest in space exploration, and especially in traveling to Mars. However, living in space can be dangerous to astronauts' health because microgravity reduces immune function. According to studies by NASA's Integrated Immune Team and Nutrition Laboratory, the concentration of cytokine molecules in blood changes in space. Cytokines are small molecules that act as ligands for immune cells. When a human body is infected by a pathogen, activated immune cells release cytokines so that other immune cells can help it in responding to the pathogen. However, when cells release too few or too many cytokines, it has an adverse effect on the body's ability to fight off infection.

By studying the effect of microgravity on dendritic cell production of cytokines, we hope to identify factors that influence cytokine production in space. Our project focuses on one common pro-inflammatory cytokine: Tumor Necrosis Factor alpha (TNF-α). We have designed an experiment to test a cellular response pathway that leads to the production and release of this cytokine. In the pathway, reception of bacterial flagellin causes dendritic cells to transcribe genes that code for cytokines. We can measure this response by conducting RT-PCR on genetic material extracted from the dendritic cells. The results of this experiment will provide valuable insight into how cytokine production changes in space.

Keywords: Space, immune, dendritic cell, cytokines

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Watch a video introduction by the authors at https://youtu.be/fEc0TnYgfIQ

Background

In recent years, there has been heightened interest in longer manned space flights. NASA has set goals for human exploration on the lunar surface and on Mars (Dunbar 2018) in the upcoming years. However, long-distance travel in space can be dangerous because of the changes it causes in the immune system. According to Dr. Brian Crucian, the principal investigator of the Functional Immune study at NASA's Johnson Space Center, "We now need to delve deeper into the immune system changes that happen in space... All the factors that change immunity on the ISS will be worse on longer missions to an asteroid or to Mars." (National Aeronautics and Space Administration 2017)

Currently, NASA has conducted research on the effect of weightlessness on rodent immune systems (Johnson 2019). Most of their research primarily focused on change in the number of immune cells and on immunological memory following vaccination. However, there are very few studies measuring production of cytokines in response to antigen or activation of innate immunity (Keeter 2019).

Cytokines are essential to immune function because they allow immune cells to communicate and cooperate to mount a defense against pathogens. The cytokine our design focuses on is TNF-α, a pro-inflammatory cytokine. We decided to study TNF-α because it is commonly produced by macrophages, but can be produced by other types of cells as well (European Bioinformatics Institute 2020). When exposed to certain antigens, human dendritic cells produce TNF-α following a signalling cascade. We plan to study how the effectiveness of this pathway is affected by weightlessness.

Systems Level

The objective of this experiment is to study the effects of microgravity on the production of cytokines by human dendritic cells in response to antigen. We plan to achieve this objective in a single experiment conducted in space, likely on the ISS. Our experimental plan consists of exposing dendritic cells to an antigen derived from bacterial flagellin and then measuring the transcription of cytokine-producing genes using RT-PCR. When we compare the results of this experiment to a normal response on Earth, we will gain insight into how cytokine production changes in space.

As ligands, cytokines function as autocrine, paracrine and endocrine messengers between immune cells. Figure 1 gives a simplified overview of how antigen triggers cytokine production, and how cytokines contribute to immune function.

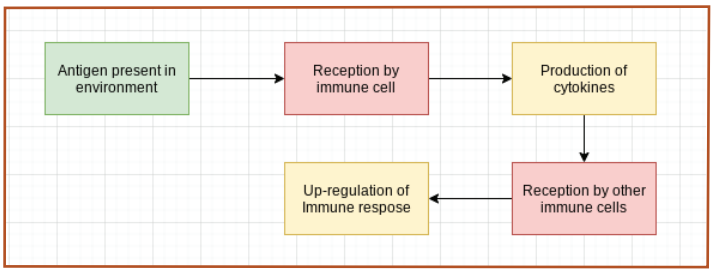

Figure 1. *Cytokine production and function*

In the first step of our experiment, we would expose samples of cultured dendritic cells to purified flagellin protein at different concentrations. In response, the dendritic cells transcribe the TNF-α gene after a cell-signalling cascade. Since H. pylori is pathogenic, we would send only the flagellin protein to the ISS to minimize the danger of infection. (Wong, Guidry, Arneson, et al. 2011)

We would then conduct RT-PCR on mRNA from our dendritic cells to determine the relative concentration of the TNF-α gene. Our negative control would be mRNA from dendritic cells that were exposed to denatured antigen protein. For our positive control, we would use a sample of mRNA containing TNF-α at the concentration that is expected on Earth. This is important to ensure that the RT-PCR yields accurate measurements and to allow for meaningful comparison of TNF-α transcription on Earth and on space. To analyze the results of our experiment, we would compare the RT-PCR of antigen-exposed cells to the negative and positive controls (Dharmaraj 2020).

Device level

In our experiment, we plan to use dendritic cells because it has been shown that dendritic cells can survive well in culture (Lodge 2017) and that they express a well-known signalling cascade following Toll-like receptor 5 (TLR5) activation by flagellin (Miao, Andersen-Nissen, Warren, et al. 2007). The flagellin we plan to use as the antigen is a chimeric fusion protein which has segments from the pathogenic bacteria Helicobacter pylori as well as Escherichia coli. Figure 2 shows how this antigen induces production of a cytokine, TNF-α (cachexin).

The cellular response pathway to the antigen is shown in the flowchart. Upon exposure to flagellin, the TLR5 receptor is activated (Ishii and Akira 2008). This causes the recruitment of the MyD88 protein, which activates the transcription factor Nuclear Factor kappa

B (Arancibia, Beltrán, Aguirre, et al. 2007), which allows the cell to transcribe the TNF-α gene (Qiu, Hu, Nesic, et al. 2004).

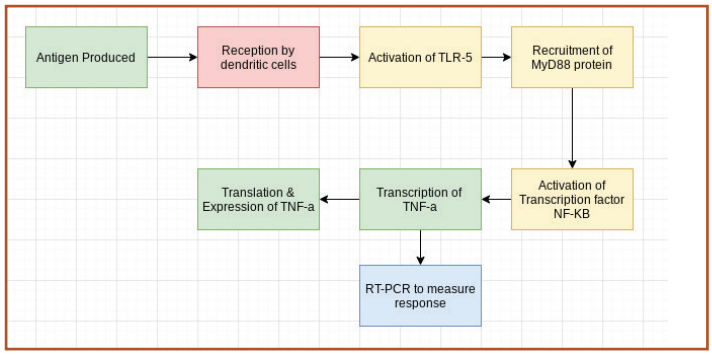

Figure 2. *Representation of cell signalling cascade in relation to experiment*

Figure 3 shows the location of the TNF-α gene on chromosome 6 of the human genome. The gene codes for the TNF-α protein, which has 233 amino acids (European Bioinformatics Institute 2020). We plan to use

Figure 3. *Image showing location of TNF-α gene*

RT-PCR to measure the rate of transcription of the TNF-α gene during our experiment.

Parts level

We plan to produce the antigen, which is a flagellin protein, using cell-free protein expression because of safety concerns associated with culturing pathogenic bacteria. Since space stations are closed spaces, culturing pathogenic bacteria poses a greater danger. (Wong, Guidry, Arneson, et al. 2011) Thus, it would be better to conduct this experiment with cell-free expression.

Cell free expression is a useful method for research that allows protein to be expressed in vitro. It is a process by which DNA can be transcribed and translated without cells but with cell extracts containing polymerase, ribosomes, and other necessary components for protein expression. This is useful because it eliminates the need for a cellular chassis that could potentially infect astronauts (Thermo Fisher Scientific 2020).

Figure 4 shows the genetic parts we plan to use in a plasmid that produces the flagellin that we plan to use as antigen. The promoter and RBS we plan to use are in the iGEM part BBa_K525998 (Drong 2011). This is a

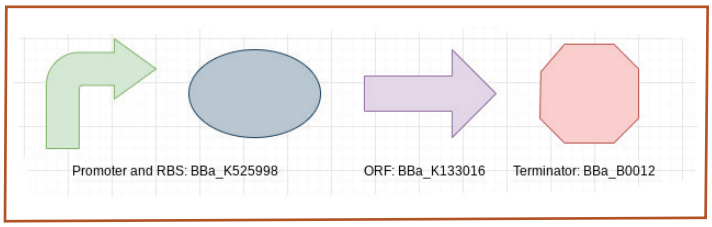

Figure 4: *Visual representation of genetic parts*

strong promoter which is transcribed only by T7 RNA polymerase. This is a preferred characteristic for cell-free expression, since the process commonly uses T7 RNA polymerase to transcribe the gene from a DNA strand (Thermo Fisher Scientific 2020).

The open reading frame is the iGEM part BBa_K133016 (Kocar 2008), which produces a chimeric fusion protein that combines antigenic segments of flagellin from H.pylori with those from E.coli. The gene codes for the entire antigen, so we plan to use a commensal serotype of E.coli to minimize danger of infection on the ISS. The resulting protein activates the TLR5 (toll-like receptor 5) receptor of a cell. This activates the signalling cascade, and transcription of TNF-α, as shown earlier. To end transcription, we placed the iGEM part BBa_B0012, a T7 terminator, at the end. (Shetty 2003)

Safety

We plan on conducting our experiment entirely in vitro to minimize the likelihood of exposure to toxins or other potentially dangerous substances. The antigen could potentially pose a danger to researchers. This is a concern especially because of the closed environment of the International Space Station. (Wong, Guidry, Arneson, et al. 2011) To eliminate the need to handle live pathogens, we designed our experiment to use cell-free expression of antigenic protein instead.

We also plan to implement precautions to prevent the cytokines from becoming aerosolized. Since the dendritic cells and cytokines are human, it would not be as dangerous as culturing pathogenic bacteria. We intend for our experiment to be performed on the ISS, where appropriate safety measures would be taken so that cells

or contaminated materials are properly contained before returning to the Earth's surface.

Discussions

Our project provides a design through which researchers can study the effects of microgravity on the innate immune system. If the results show that cytokine transcription decreases in space, this could help us compensate for the changes by changing some aspects of astronaut diet or lifestyle to promote more immune cell activity. On the other hand, if cytokine production actually increases in space, astronauts could counteract it with anti-inflammatory treatment.

If the concentration of TNF-α was lower than the positive control, this would indicate that cytokine production decreases in space. If the concentration of TNF-α was greater than the positive control, it would indicate that cytokine production is too high in space. If we had a null result, in which TNF-α concentration is not significantly higher or lower in space, it could mean that our method of collecting data was flawed. In this case, we could repeat the experiment with a different protein. This could be an antigen that activates the same MyD88 pathway, or it could be one that causes production of a different cytokine.

A null result could also indicate that the changes in cytokine production occur after transcription. This provides an opportunity to further study changes in the production of cytokines during translation, or transport in vesicles through techniques such as SDS-PAGE or enzyme-linked immunosorbent assays.

The biggest challenge in this project is to ensure the safety of the researchers. Using cell-free expression would eliminate the need to culture dangerous bacteria in space. However, before conducting the experiment, it is necessary to study the antigen protein further to understand if it could have an adverse effect on researchers. If the antigen was found to pose a danger, protective measures could be implemented or a different protein could be utilized as antigen.

This design has a lot of potential because it could be used as a basis for similar experiments in the future. It could be replicated with other immune cells or with other antigens to characterize production of a number of different cytokines. This would result in a comprehensive understanding of how weightlessness impacts different aspects of the innate immune system. Having a better understanding of this facet of immune system function could contribute to improving astronauts' safety and health in space.

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