Gene-Expression Differences in *Streptococcus pyogenes* Strains that Cause Infective Endocarditis

Victoria Rios[1], Yvianna Rodarte [1], John Rovin [1], Katherine Kirtley [1,2], Rebecca Bossie [1,2], Dr. Jacen Moore [1,3]

[1] Work With A Scientist Program [2]College of Education [3] College of Health Sciences, Clinical Laboratory Science Program

Research Question

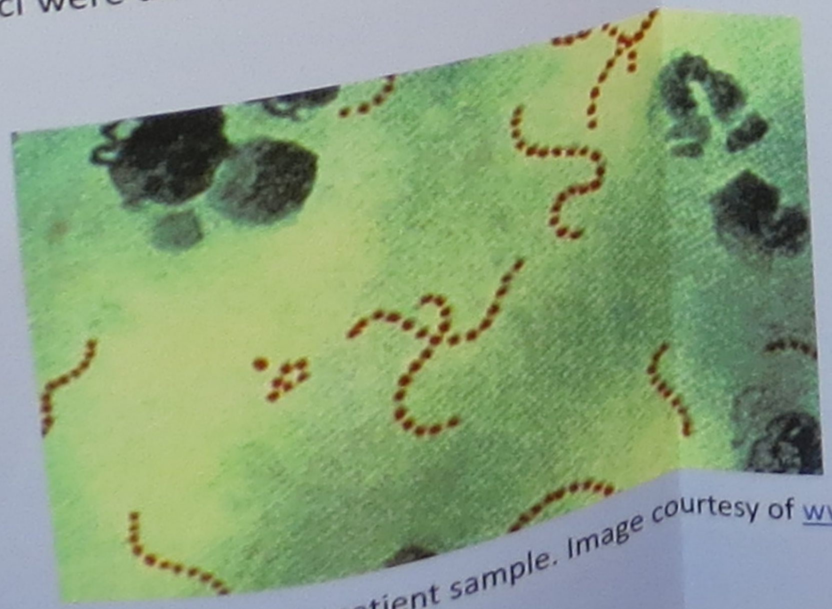
Are there any particular virulence genes which are under-expressed or over-expressed in *Streptococcus pyogenes* that influence attachment of the bacteria to tissue of the inner surface of the heart upon entering the bloodstream, leading to Infective Endocarditis?

Abstract

Infective Endocarditis is a disease wherein bacteria that have entered the blood infects the endocardium, the inner tissue of the heart. Patients or individuals with heart conditions are most at risk of contracting the disease [1]. This experiment will attempt to identify why the pathogen *Streptococcus pyogenes* causes Infective Endocarditis. We plan to apply gene-expression testing to discover if the reason for the pathogen's attachment to the inner lining of the heart is related to the expression or over-expression of particular genes. We propose to obtain a sample that includes an isolate of bacteria from a patient diagnosed with Infective Endocarditis due to *Streptococcus pyogenes*. If we obtain such a sample, we will then attempt to compare the gene-expression differences amongst the two isolates. This will therefore, allow us to see if there is evidence of an association between gene expression and the nature of *Streptococcus pyogenes* [Personal Communication]. If our hypothesis is correct, this data will allow us to better understand the manner in which Infective Endocarditis develops in the heart so that future researchers and medical physicians may efficiently diagnose and treat the disease and possibly find preventions that would be more effective in the future.

Introduction

- Our bodies have bad bacteria which occupy an individual's body and may take advantage of momentary vulnerabilities in her/his immune system to cause disease [3].
- Dental procedures and subsequent dental trauma are some of the many possible means by which dangerous pathogens are introduced into the bloodstream, known as bacteremia [4].
- Bacteria circulating in the blood then have the potential to attack the heart, sometimes as an intravascular infection known as Infective Endocarditis (IE) which is also referred to as Bacterial Endocarditis (BE).
- Streptococci were also listed in 24.7% of Infective Endocarditis cases in the United States [5].



Streptococcus pyogenes in patient sample. Image courtesy of www.healthmap.org

Methods

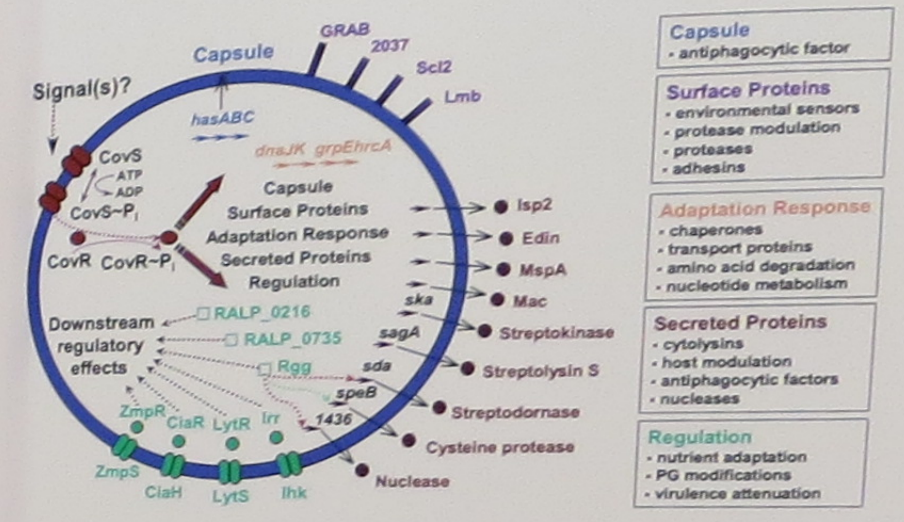
- *S. pyogenes* will be cultured for approximately three days
- *S. pyogenes'* RNA will then be isolated.
- Employing reverse transcription, we will produce cDNA from our isolated RNA.
- The cDNA will be labeled with fluorescent probe for hybridization.
- We will apply gene expression testing (hybridization) using labeled cDNA to obtain fold-change expression information.
- We will collaborate with a Bioinformaticist who will use software programs to perform a gene-expression analysis.

Materials

- Microbiology plates (5% sheep's blood and "chocolate"-lysed blood)
- Micropipettes and tips
- Vortexers (for mixing)
- Centrifuge (for separating)
- Polymerase Chain Reaction reagents
- Spectrophotometer
- RNA Isolation Kit from Quiagen
- RT-PCR Kit from Invitrogen
- *S. pyogenes* bacteria
- Commercially available gene expression chip(s)
- Collaborate with Bioinformaticist for chip analyzer and software
- Agarose gel electrophoresis unit and power pack
- Buffers
- Ethidium Bromide
- Molecular Weight marker

Timeline

	Projected Timeline for Experiment
Week #	Task(s) for the week
1	Firstly, we will learn the methodology and each of the techniques required to properly and safely carry out the experiment.
2	Next, we will grow the *S. pyogenes* on 5% sheep's blood. After one to three days of culturing we should have viable colonies of *S. pyogenes*. From these colonies, we will isolate the RNA of the bacterium and apply reverse transcription to produce cDNA for analysis.
3	We will then use the cDNA in gene-expression testing to gather basic data about which genes are expressed and at what level, from which a pathway analysis will be performed to gather basic data.
4	We will also attempt to obtain an isolate of bacteria from a patient diagnosed with infective endocarditis due to this pathogen. If we are successful, we will then attempt to compare the gene-expression differences amongst the two isolates.
5	In working with a Bioinformaticist we will identify which genes, based on fold-expression changes, may be involved in the targeting of heart tissue.
6	Lastly, conclusions will be drawn from the previous weeks' results and the final proposal will be written up.



Capsule
- antiphagocytic factor

Surface Proteins
- environmental sensors
- protease modulation
- proteases
- adhesins

Adaptation Response
- chaperones
- transport proteins
- amino acid degradation
- nucleotide metabolism

Secreted Proteins
- cytolysins
- host modulation
- antiphagocytic factors
- nucleases

Regulation
- nutrient adaptation
- PG modifications
- virulence attenuation

Streptococcus pyogenes genes and virulence factors. Image courtesy www.pnas.org

Expected Outcomes

To find particular virulence genes/factors whose degree of expression provides novel insights and as to why the *Streptococcus pyogenes* pathogen specifically attaches to the inner tissue of the heart. We may identify a particular gene or group of genes which influence this behavior in *Streptococcus pyogenes*.

References

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