Finding a Link Between Impetigo and Rheumatic Heart Disease

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Finding a Link Between Impetigo and Rheumatic Heart Disease

Lindsey Meservey, Thomas Knapp, Nathan Mella, Tyler Heaton, Tanner Heaton, Jonathon Hill, Paul Savage

HYPOTHESIS: Although it is traditionally believed that rheumatic heart disease originates only from strep throat infections, we hypothesize strep infections of the skin to be the primary culprit behind the prevalence of rheumatic heart disease in tropical countries such as Samoa.

ABSTRACT
Rheumatic heart disease (RHD) causes over 300,000 deaths annually, most of them in third world countries. Decades of research confirm that untreated strep throat infections (caused by group A strep, or GAS) can trigger an autoimmune response that leads to RHD. However, despite increased worldwide attention toward treating strep throat, RHD rates can continue to increase, especially in tropical countries like Samoa. We hypothesize that GAS infections of the skin, commonly called impetigo, are also responsible for causing RHD in Samoa. We are teaming with the Samoa Rheumatic Relief Internship program to test this hypothesis. We will screen children for RHD and take throat, leg sore, and mat swabs. We will then analyze these samples to see if and which strains of GAS are present. We predict that the correlation between the presence of GAS in leg wounds and RHD will be as high as that as between GAS in the throat and RHD. We also expect to see that strains of GAS in leg wounds will be similar to those found on the throat and expect to find that certain strains of GAS are more strongly correlated with RHD, consistent with previous research.

INTRODUCTION
Rheumatic heart disease (RHD) is the result of an autoimmune attack of the heart valves. Previous research has shown that untreated strep throat infections can cause RHD. Despite treatment of strep throat, however, RHD rates remain rampant in many regions of the world, especially tropical islands. In Samoa, for example, 8% of elementary school students have RHD. Samoans have a low rate of strep throat, but an extremely high rate of impetigo, a skin infection caused by the same species of strep, group A strep (GAS). Researchers have shown correlations between impetigo and RHD in other regions of the world. Thus, we hypothesize that RHD is also caused by GAS infections of the skin. We will test this hypothesis by correlating rates of GAS skin infections with rates of RHD in Samoan children.

TRADITIONAL & NOVEL HYPOTHESES

Traditional Model:
1. Strep colonies the throat, causing antibodies to be made.
2. If the infection is not treated, these antibodies then attack the heart valves, creating acute rheumatic fever (ARF).
3. ARF results in permanent damage to the heart valves, or rheumatic heart disease (RHD).

Novel Hypothesis:
1. Strep colonies the skin, causing antibodies to be made.
2. If the infection is not treated, these antibodies then attack the heart valves, creating acute rheumatic fever (ARF).
3. ARF results in permanent damage to the heart valves, or rheumatic heart disease (RHD).

RHEUMATIC RELIEF SAMOA
The Rheumatic Relief Internship Program combines undergraduates, cardiologists, and public health experts to diagnose and treat RHD in Samoa. Members of this program visit Samoa yearly to diagnose new cases of RHD and teach children how to prevent strep infections. We will accompany the Rheumatic Relief program to Samoa to take samples.

METHODS
• Cardiologists from the Rheumatic Relief program will use echocardiograms to test Samoan children for RHD.
• For any children testing positive for RHD, we will take swabs of the throat and any skin wounds.
• We will plate the swabs and identify any colonies of group A strep.
• We will extract DNA from group A colonies and send this DNA back to the lab.
• In the lab, we will use the standard emm-typing protocol to determine the strains of strep present.
• We correlate strains and locations of group A strep with RHD.

DISTINGUISHING GROUP A STREP
Other species of bacteria may show similar phenotypes to strep. For example, Staphylococcus aureus, which is very common in skin flora, also does full beta hemolysis. Thus, we tested isolation of GAS colonies by plating swabs of skin, carpets, and throat flora with and without GAS. We were successful at isolating strep from all cultures containing strep.

OPTIMIZING PLATE CULTURES
Hemolysis (lysing blood cells) is a key feature in identifying GAS. GAS displays complete hemolysis within 24 hours when grown at 37°C in anaerobic conditions (i.e. 5% CO2). However, as we will be unable to bring an incubator or CO2 tanks, we need to optimize plate cultures at Samoa’s atmospheric temperature, which is about 30°C. Using parallel to promote anaerobic conditions, we show that beta hemolysis occurs within 48 hours at 30°C.

OPTIMIZING STRAIN IDENTIFICATION
After confirming presence of GAS, we identify strains by sequencing the emm region of the GAS genome. To do this, we extract DNA from the GAS and then perform PCR and Sanger sequencing. We found that extracting DNA with Qiagen’s UltraClean Microbial Kit yielded the best results PCR and sequencing (see gel and sequence data on right). The kit requires a pellet of bacteria, which must be grown in liquid culture in Samoa. We tested two different mediums (TSB and BHI) at 30°C and found that cultures grown in BHI with a tightly closed lid yielded the most DNA (see graph on right).

FUTURE DIRECTIONS
After determining a correlation between impetigo and RHD, we hope to organize clinical trials in Samoa to test causality. Low-cost topical drugs such as bactroban are extremely effective at killing GAS. Thus, we would treat skin wounds with bactroban and expect to see reduced rates of RHD. We hope these studies will inform health care policies in Samoa and other regions of the world. Currently impetigo is usually left untreated in many countries; however, if impetigo is responsible for causing RHD, then more could be done to treat impetigo infections and prevent cases of RHD.

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