HELPING TO DEVELOP A GONORRHOEA VACCINE

DR CYNTHIA NAU CORNELISSEN IS A MICROBIOLOGIST WHO RUNS HER OWN LABORATORY AT **GEORGIA STATE UNIVERSITY** IN THE US. HER CURRENT RESEARCH IS FOCUSED ON UNDERSTANDING MORE ABOUT GONORRHOEA. THE FINDINGS COULD ONE DAY LEAD TO A VACCINE FOR THIS SEXUALLY TRANSMITTED DISEASE

TALK LIKE A MICROBIOLOGIST

GONORRHOEA – a sexually transmitted disease

NEISSERIA GONORRHOEAE – a bacterium that causes gonorrhoea

PATHOGEN – an organism that causes disease to its host

OBLIGATE HUMAN PATHOGEN – a pathogen that must infect a human and cannot infect other animals and does not exist freely in the environment

GLYCOPROTEIN – a protein with a carbohydrate – or sugar – attached to it

TRANSFERRIN – glycoproteins found in vertebrates which bind to and consequently mediate the transport of iron through blood plasma

BACTERIAL PATHOGENESIS – the process by which bacteria infect and cause disease in a host

BACTERIAL PHYSIOLOGY – a scientific discipline that concerns the life-supporting functions and processes of bacteria

CHELATION – a type of bonding of ions and molecules to metal ions

METAL-BINDING PROTEINS – proteins or protein domains that chelate a metal ion

Gonorrhoea is a sexually transmitted disease caused by the bacterium *Neisseria gonorrhoeae* (*N. gonorrhoeae*). The Centers for Disease Control and Prevention estimates that approximately 1.6 million new gonococcal infections occurred in the United States in 2018, and more than half occur among young people aged 15-24. The World Health Organization puts the global figure at 100 million cases every year.

The disease can infect both men and women and, like all sexually transmitted infections, cases have dramatically increased over the past decade or so. There are some potentially serious implications of contracting gonorrhoea, such as pelvic inflammatory disease, ectopic pregnancy, and infertility – all of which can have significant implications for the fertility of the younger generation.

It is with these issues in mind that Dr Cynthia Nau Cornelissen is conducting research in her laboratory at the Institute for Biomedical Sciences at Georgia State University in the US. Cindi (Cynthia) is a microbiologist who leads a team attempting to characterise the virulence factors that enable *N. gonorrhoeae* to cause infection. The findings from her team's research could lead to the development of a vaccine that prevents individuals from contracting gonorrhoea.

THE NEED FOR A VACCINE

Like many bacterial infections and diseases, the overuse of antibiotics has led to antibiotic resistance in gonorrhoea. The genetic flexibility of the organism has given rise to an infection referred to as super gonorrhoea, which is when *N. gonorrhoeae* develops a high level of resistance to ceftriaxone, the antibiotic normally used to treat the infection. Such resistance has led to a scenario where untreatable gonorrhoea could soon become a reality. With antibiotic resistance reaching critical levels, a better way to approach this problem is by developing a preventative vaccine to protect against gonorrhoea disease. "Researchers have been trying to develop vaccines against this pathogen for decades, but the variable surface structure of *N. gonorrhoeae* has thwarted these efforts. The gonococcus has been referred to as a chameleon because almost all of its surface structures are subject to high-frequency variation. This variation means that the human immune response cannot keep ahead of the variation, plus these surface structures are so variable among different strains, that their use in a vaccine would not be universally protective," explains Cindi. "So, our approach has been to try to identify proteins on the cell surface that are necessary for the pathogen to grow and cause disease but that are also not subject to high

frequency variation. Nutrient transporters fulfil these requirements, so we are pursuing them as potential vaccine components."

Cindi has been studying the physiology of bacteria, i.e. how bacteria develop and flourish, since she was an undergraduate at college. All of her training has led her to pursue the hypothesis that blocking nutrient transport to interfere with bacterial growth will be the key to prevention and perhaps lead to the development of novel treatments, too.

INVADING PATHOGENS

Nutritional immunity is a form of innate immunity. The human host goes to great lengths to hide metals from invading pathogens. This is an attempt to thwart the microbes because these metals are critical to microbial replication and pathogenesis. Human hosts do so by making a whole suite of proteins that bind to metals with very high affinity. "Most pathogens try to overcome this metal restriction by producing low molecular weight molecules called siderophores. These metal cages can also bind metals, mostly iron, with very high affinity," says Cindi. "In this way, the pathogens can compete with the human metal-sequestering proteins and overcome nutritional immunity."

METAL PIRACY

Interestingly, *Neisseria gonorrhoeae* does not have the genes that enable it to produce siderophores. Instead, it can use siderophores produced by other bacteria, such as *E. coli*. But *N. gonorrhoeae* also uses the metal binding proteins directly, in a process the team has called metal piracy. "They actually hijack the human metal binding proteins by directly binding these proteins to bacterial outer membrane receptors," explains Cindi. "When this binding event happens, the bacteria can literally extract the metals from the host proteins, internalise the iron, and then release the host protein back into solution without breaking it down or changing it in any way. This pathogen is very stealthy."

IRON TRANSPORT

Humans produce a series of nutritional immunity proteins. Some of these bind to iron and some bind to other important transition metal proteins, such as zinc and manganese. Examples of these proteins are transferrin and lactoferrin, which bind iron, and some S100 proteins, which bind to zinc and manganese. The outer membrane proteins produced by *N. gonorrhoeae* can directly bind to these human metal sequestering proteins and relieve them of their metal cargo.

IN THE LAB

Cindi and the team use a range of genetic techniques, including bacterial transformation and PCR, also known as polymerase chain reaction. (A modified form of PCR called reverse transcriptase-PCR, or RT-PCR, is used to detect coronavirus.) The team also uses fluorescent techniques, including confocal microscopy and it does a lot of gene expression studies using techniques like RT-qPCR and RNA-seq. Fascinatingly, the lab also grows *N. gonorrhoeae* – a necessity for a team intent on finding a means of thwarting it. This can be challenging because *N. gonorrhoeae* is known for being quite picky about the conditions it needs to grow; the team cultures it in media that are very rich in nutrients, and at human body temperature in an environment that includes 5 percent CO_2.

SUCCESSES

When Cindi was a postdoc, she and the team showed that a *N. gonorrhoeae* mutant that could not produce the transferrin receptor was unable to cause experimental infection in human males. Since then, Cindi's laboratory has shown that these nutritional immunity protein receptors are exquisitely specific for the human forms of the metal binding proteins. "They do not recognise any other forms of these proteins, including those of mice or other mammals. This means that animal modelling with this pathogen is exceedingly difficult," explains Cindi. "We have also shown that these outer membrane receptors are very well conserved, critical to survival and are potential vaccine or drug targets in the future."

THE NEXT STEPS

Cindi and the team are collaborating with several groups that do vaccine studies to test whether outer membrane proteins can protect against *N. gonorrhoeae* infection. Such experiments are challenging because the receptors do not bind to the mouse forms of these proteins; the team has to produce transgenic mice that produce the human forms of the nutritional immunity proteins. It is a long process, but one that will reap significant rewards if successful. Incredibly, Cindi was once told she would never make it as a scientist! We can see how far she has come and how determined she is to succeed at this challenging task.

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FIELD OF RESEARCH

Microbiology and Immunology

RESEARCH PROJECT

Characterising virulence factors that enable the sexually transmitted pathogen, *N. gonorrhoeae*, to cause infection, with the aim of developing a gonorrhoea vaccine

FUNDERS

National Institute of Allergy and Infectious Diseases, National Institutes of Health

ABOUT MICROBIOLOGY

Microbiologists study micro-organisms like bacteria, viruses, fungi and algae, which means the field is enormously complex and diverse. For those that achieve the necessary qualifications to study microbiology, you could just as easily end up working for a company as you could work as an academic in a laboratory – it all depends on which area you want to head into.

Because of the diverse nature of the work, it is difficult to sum up the field in a nutshell. In Cindi's case, she is focused on developing experiments that could one day lead to a gonorrhoea vaccine, but you could find yourself working on the study of nematodes or parasites. Irrespective of the specific area that any given microbiologist is focused on, the principles are the same and involve studying extremely small examples of life.

THE REWARDS

Above anything else, Cindi is excited to go to work every day. "I love doing and teaching microbiology. I have taught all types of students at all levels about the wonders, and perils, of microbiology. I have given lectures to elementary, middle and honours high school students," says Cindi. "I taught medical and dental students for decades, in addition to graduate students. I love training the next generation and microbiology is certainly my passion."

THE NEXT GENERATION

The COVID-19 pandemic has certainly brought the importance of microbiology into sharp focus. "I think the last two years have taught us that pathogens will continue to emerge, and that the next worldwide pandemic could be right around the corner," explains Cindi. "Microbes are always evolving to become more effective pathogens, even if that means they are more transmissible, but not more virulent. Or maybe it means they change to escape existing immune responses, whether vaccine-mediated or due to natural infection. We can never turn a blind eye to microbial evolution. I also think we will have to do a much better job of overcoming vaccine hesitancy."

A PASSION FOR MENTORING

Ultimately, the reason for Cindi's passion in this area is that we need more STEM-trained citizens. "The next generation of scientists can go on to do many important things in society, including communication, administration, clinical microbiology, industry, academia, or even enter politics!" says Cindi. "We need more scientific literacy at large and training an outstanding next generation of scientists is the best way to get there."

EXPLORE A CAREER IN MICROBIOLOGY

Microbiology societies play an important role in helping students develop their career.

Check out the American Society for Microbiology, which features a wide range of resources for budding microbiologists: **asm.org/Articles/2018/November/Careers-in-Microbiology-and-the-Microbial-Sciences**

The Microbiology Society also provides a wealth of interesting careers resources: **microbiologysociety.org/members-outreach-resources/careers-resources.html**

According to Payscale.com, the average salary for a microbiologist in the US is $55,000.

PATHWAY FROM SCHOOL TO MICROBIOLOGY

Cindi believes it is critical that students have a firm background in chemistry, especially biochemistry and cell biology, to be successful in microbiology. "Other sciences are obviously helpful too, but some subjects are more critical than others," she explains. "I would also say that knowing something about coding and computer science will be valuable. Bioinformatics has become a huge part of the microbial sciences."

CINDI'S TOP TIPS

01 Never listen to any people that tell you cannot do science. I was told that upon applying to college and it only served as motivation to work harder!

02 Try your best to put everything you have into your education and career – it is the best way of ensuring you will be successful.

03 If you are interested in pursuing a career in microbiology, do your best to join a society. I have been an active member of the American Society for Microbiology for decades. They provide conference and workshop opportunities for all levels of microbial scientists.

HOW DID CINDI BECOME A MICROBIOLOGIST?

I was always very immersed in school. I gravitated towards biology, chemistry and physics. I was lucky in that I also always read a lot. Interestingly, in my freshman year in college, I took a mandatory composition course. My professor told me that I would never write a flowery novel but that I would be very good at scientific writing. That advice and realisation served me well in my chosen career!

I think my upbringing in a very small town in southern Illinois spurred me to work really hard to get into and then through college. I very much wanted a career in science and knew that it would take a college degree, at least, to get me there. There was always the expectation that I would go to college when I was growing up. I did well in high school and got a partial scholarship as a result. I always felt it was up to me to work as hard as I possibly could to get where I wanted to go. Nothing would be given to me.

I evolved into realising that I could be a scientist. I think I always wanted to be one but didn't have any role models. I took all science courses in college – biology, chemistry, physics, math and computer science – , but when I started working as a dishwasher in a microbiology lab, I learned what microbiology was all about. After that, I declared microbiology as my major, and did a senior research project in the same lab. By this time, I was also doing all of the lab ordering and maintenance. In retrospect, these experiences really helped me in setting up my own lab. I ended up acquiring both a MSc and PhD in microbiology but wasn't sure that I wanted to be a professor. My postdoctoral mentor allowed me the freedom to do research, have a family and get my first grant. From that point forward, it didn't matter if I didn't have any role models. I decided I wanted to be a role model for others; thus, my desire to help mentor and train the next generation.

I overcome scientific obstacles by facing them, asking questions and reading the literature. Switching off from work is a different matter – it's about a work-life balance. When my children were small, spending time with them was my only 'hobby' outside of work. I have a supportive husband who helped me juggle all the parental responsibilities. After the kids grew up, my husband and I cultivated new hobbies. We sailed for years on the Chesapeake Bay when we lived in Virginia. Now that we are in Georgia, we have other fun 'switch off' activities, such as swimming, hiking and camping. These activities are very important to staying balanced and not burning out.

What are my proudest achievements? Being awarded my first NIH grant, being promoted to Full Professor, being the first author on two textbooks and one set of microbiology flash cards, being awarded an NIH programme project grant, being inducted as a fellow into the American Academy of Microbiology, and being promoted to Associate Director of the Institute for Biomedical Sciences at GSU! I am also enormously proud of all of my trainees and students.