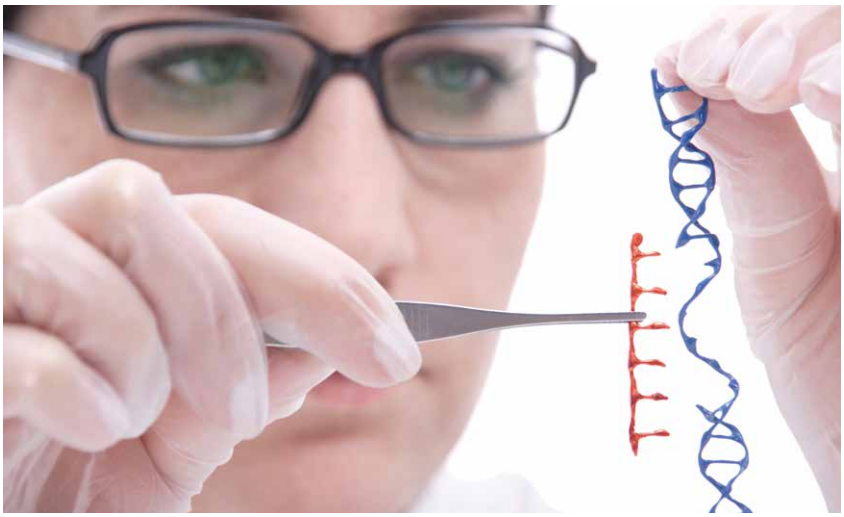
Good Bacteria Gone Bad

Professor Hannah M. Wexler

Scientia

GOOD BACTERIA GONE BAD

Bacteria within our gut play an essential role in breaking down our food, but when they escape to a new environment some can turn nasty in order to survive. **Professor Hannah Wexler's** lab at the Greater Los Angeles Veterans Health Care System (GLAVAHCS) has been investigating what mechanisms *Bacteroides* gut bacteria use to survive when they inadvertently escape and how we can stop them.



The Transition from Friend to Foe

The average human body contains ten times as many bacterial cells as human cells, and up to 100 times as many bacterial genes! It is no wonder then that bacteria have important roles in diverse human bodily functions, including immunity and digestion. The gastrointestinal (GI) tract is where most of these microorganisms are found, with a staggering 10^{11} per gram in the colon. The bulk of these bacteria are anaerobes and don't require oxygen to survive. A quarter of these anaerobes are *Bacteroides*, an ancient family of microorganisms that colonises the human gut during birth and is established after ten days.

The relationship between humans and *Bacteroides* is complex, but mostly mutualistic. In exchange for room and board in our colon, these bacteria use their vast array of digestive enzymes to break down complex carbohydrates known as polysaccharides, liberating simple sugars, volatile fatty acids and vitamins, which make up a significant proportion of our nutrient needs.

Furthermore, studies have shown that *Bacteroides* contribute to the early development of our immune systems and prevent colonisation by harmful pathogenic bacteria. They are involved in our immune system in antibody development, they stimulate colon immune cells to produce antimicrobial proteins and peptides and they stimulate the development of CD4+ T-cells, the memory cells in our immune armament that recognise pathogenic bacteria and direct an appropriate immune response.

However, the metabolic adaptability afforded to *Bacteroides* by a large genome is also crucial to its ability to transition from friend to foe when it ends up outside the colonic niche. In situations where the integrity of the GI tract is compromised, such as in appendicitis or from surgical or traumatic wounds or cancer, certain species of *Bacteroides* can adapt by 'turning on' a range of genes involved in pathogenesis known as virulence factors.

When turned on, these genes produce proteins that help the bacterium to adhere to and feed off host tissues and protect them from immune cell attack. They can also uptake and spread a range of mobile genetic elements that are common within the packed gut environment, and that can confer resistance to antimicrobials, making infections of this type extremely dangerous and difficult to treat.

Surgical site infections are the most common type of healthcare associated infection, with 157,500 identified by the Center for Disease Control in 2011. The consequences of acquiring drug resistant surgical site infections can include increased pain and suffering, increased hospital stay and increased mortality; with around 8000 deaths a year and a 2–11-fold higher risk of death.

Bacteroides fragilis is the most common anaerobic causative agent and is responsible for 17% of organ space surgical site infections. It is also the main anaerobic bacterium that causes blood infections and is implicated in other serious infections, including intra-abdominal and brain abscesses. Professor Hannah Wexler and her team at GLAVAHCS have been working for over 30 years on characterising the virulence and antimicrobial resistance mechanisms that allow *Bacteroides fragilis* to change from a friendly commensal organism to a deadly pathogen. Development of new approaches to therapy requires that we understand how *Bacteroides fragilis* makes this transition.

‘An antimicrobial should be closer to a scalpel than a sledgehammer.’

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The Commensal Chameleon

Bacteroides fragilis has two main traits that allow it to switch from good to bad – an ability to easily incorporate genes shared by other bacteria and an ability to turn specific genes ‘on’ or ‘off’ as needed. Combined, these traits allow these bacteria to exploit new nutrition pathways, protecting themselves from toxic substrates, and changing the molecules expressed on its surface. This ‘commensal chameleon’ is the perfect opportunistic pathogen.

Bacteria take up new genes by horizontal gene transfer from related or unrelated bacteria. This process is responsible for the global dissemination of both virulence and resistance genes that undermine the usefulness of most antimicrobials. Amongst commensal bacteria, *Bacteroides fragilis* has a well-established role as a ‘resistance reservoir’, because it is extremely good at incorporating genes from others of its kind as well as ‘foreign’ genes into its extremely plastic genome by this process of gene transfer.

However, *Bacteroides fragilis* also possesses numerous systems for destroying disadvantageous or harmful invading DNA molecules. Restriction modification systems degrade foreign DNA and are controlled by molecular switches on the genome known as invertible promoters, which are turned ‘on’ or ‘off’ as needed for adaptation to a particular niche environment. Professor Wexler recently demonstrated that *Bacteroides fragilis* strains possess multiple types of the recently described CRISPR-Cas systems, bacterial adaptive immune systems that recognise, remember and destroy harmful invading DNA, such as bacterial viruses.

From her decades of studying *Bacteroides fragilis*, Professor Wexler hypothesises that an intricate balance between DNA uptake and destruction, controlled by the changing availability of resources in the gut environment, is what has shaped its adaptability.

Inside and Out – Early Discoveries

In their early work, Professor Wexler’s group was the one of the first to describe the outer membrane proteins in these bacteria. Gram negative bacteria such as *Bacteroides* have a double layered cell membrane. The outer bacterial membrane acts like a suit of armour to protect the bacterium from harmful agents, while the inner membrane acts like a selectively permeable skin, separating the inside from the outside.

The Wexler lab identified the OmpA protein, which can be implicated in virulence in other bacteria, as the major outer membrane protein and critical for maintaining cell structure. Porins are another class of membrane proteins that act as channels controlling the diffusion of specific molecules into the cell. The Wexler team described the porin protein complex Omp200, and found that it is involved in acquiring and utilising polysaccharides, the major food sources in the large intestine.

Later in 2004, publication of the *Bacteroides fragilis* genome sequence revealed dozens more outer membrane proteins yet to be studied. It is likely that some of these are porins that are produced at sites of infection to facilitate the uptake of host cell surface proteins and lipids. The Wexler lab also studied the role that porins play in resistance to certain antimicrobials, by preventing their transport into the bacterial cell.

Pumping Out the Drugs

Sometimes an antimicrobial drug can get inside the cell, but some bacteria have a way of pumping it out again before it can act. Multidrug resistant aerobic bacteria were known to use efflux pumps, inner membrane spanning proteins that use chemical energy to expel the antimicrobial agent from the cell. Professor Wexler’s group was among the first to identify these systems as an important multidrug resistance mechanism in anaerobic bacteria of clinical importance.

Working with their Japanese colleagues, the team identified 16 efflux pumps responsible for multidrug resistance in *Bacteroides fragilis*. The team went on to validate the importance of the efflux pumps in conferring multidrug resistance to bacteria that cause clinical infections, and explained how the pump's 'on' switch is triggered by the presence of antimicrobials in the environment.

Pre-packaged Pathogenicity and Virulent Families

The Wexler Lab studies *Bacteroides fragilis* strains that are responsible for serious, multidrug resistant clinical infections around the world. Often, a bunch of genes useful for pathogenicity, such as resistance genes and virulence factors, are shared together on large mobile genetic elements. Professor Wexler's team has identified numerous types of these mobile pathogenicity packages that *Bacteroides fragilis* has acquired, which contribute to its opportunistic pathogenicity.

In a strain causing a resistant appendicitis infection, they identified a new type of resistance gene called *nimJ* and investigated its role in reducing the effectiveness of metronidazole, the first line antimicrobial for *Bacteroides fragilis* infections. Upon further investigation, they discovered that *nimJ* was carried on a large mobile genetic element, which they called CTnHyb – Conjugative Transposon Hybrid. This element contained several other antimicrobial resistance and virulence genes as well as a hybrid mosaic of 'foreign' mobile elements identified as originating in unrelated bacteria. Transfer of CTnHyb to a non-pathogenic *Bacteroides fragilis* strain was demonstrated in the laboratory.

Another transfer experiment revealed the horizontal gene transfer of a large chromosomal segment from the pathogenic *Bacteroides fragilis* strain to a lab strain. The genes transferred replaced a large segment of the recipient chromosome and included genes involved in nutrient adaptation, immune recognition, host interaction and restriction modification systems that attack foreign DNA. This demonstrated the potential that this form of gene transfer has for facilitating rapid environmental adaptation.

A systematic study of the published genomes of 110 strains of *Bacteroides fragilis* undertaken by Professor Wexler's team suggested that virulent strains causing resistant blood infections belonged to a distinct subgroup. More recently, they found that *Bacteroides fragilis* containing blood isolates contain distinct CRISPR-Cas systems, now known to be acquired immunity systems in bacteria and important in controlling horizontal gene transfer and host interactions and that the differences in the CRISPR-Cas system are evident in these 'blood' isolates as well.

Interrogating Tolerance and Persistence

Antimicrobial resistance is amongst the most important factors for pathogenicity in bacteria. *Bacteroides fragilis* is an important multidrug resistant pathogen – despite its relatively modest published resistant rates, it is a tenacious microbe that often defies antimicrobial treatment. Professor Wexler is currently surmising that this bacteria's frequent implication in chronic multidrug resistant surgical site infections may be due in part to an ability to survive antimicrobial treatment through tolerance and persistence mechanisms that are distinct from resistance.

Antimicrobial tolerance can occur when protein production is switched off by a molecular trigger, leading to dormancy and the formation of what is known clinically as a 'persister', a cell that can survive treatment and then reanimate to reinstate a chronic infection. Professor Wexler is interested in how this system works in *Bacteroides fragilis*. She plans to use the latest systems biology approaches (computational modelling of complex biological systems) to study the 'tolerome' and 'persistome', the genes and proteins involved in tolerance and persistence, of pathogenic *Bacteroides fragilis* strains isolated from blood.

Swapping the H-bomb for the Surgical Blade

A problem with most antimicrobials is that they target a molecule or pathway present in all bacteria. The result is like an atomic bomb exploding in the gut, which wipes out all but the most resistant bacteria. As well as impacting digestive health, this contributes to the evolution and spread of resistance mechanisms in bacteria, as strains that have these mechanisms are at a competitive advantage. Indeed, the number of effective antimicrobials is dwindling by the year and there are genuine fears in the medical community that this will herald a return to the pre-antimicrobial era, when the simplest surgery or infection carried a great risk of mortality.

Professor Wexler believes that a more targeted approach is preferable and that, 'an antimicrobial should be closer to a scalpel than a sledgehammer.' She hopes to investigate if the same CRSIPR-Cas systems that bacteria use to protect them from invading DNA can be re-engineered as bespoke bactericidal weapons, designed to selectively destroy bacteria carrying specific pathogenicity genes, such as the ones she hopes to identify in the *Bacteroides fragilis* tolerome/persistome. This bactericidal blade could also be repurposed to chop up the genes themselves, thereby restoring the effectiveness of antimicrobials and reducing the global spread of resistance.

Meet the researcher

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Professor Hannah Wexler attended UCLA as an undergraduate and obtained her PhD in microbiology at New York University in 1979. She went on to pursue postdoctoral research at the University of California Los Angeles (UCLA) and in 1982 she joined Dr Sydney Finegold's group at the Greater Los Angeles Veterans Affairs Health Care System (GLAVAHCS) as director of the Wadsworth Anaerobe Laboratory. In 1986, she became an independent Principal Investigator and formed the Wexler Lab in the Research Division of GLAVAHCS. She is Adjunct Professor of Medicine at the UCLA School of Medicine. She served as Chair of Division A of the American Society of Microbiology in 2011 and Councilor of Division A in 2012 and holds a Career Scientist Award from the Department of Veterans Affairs. She is a member of the American Academy of Microbiology, the Anaerobe Society of the Americas and the Infectious Diseases Society of America and the American Society for Microbiology. She served on the Working Group for Anaerobes for the Subcommittee on Antimicrobial Susceptibility Testing of the Clinical Laboratory Standards Institute (CLSI) for almost thirty years and was one of the primary authors of the M11 Standard for Determination of Susceptibility for Anaerobic Bacteria and its subsequent versions for more than twenty years. Her oft-quoted 2007 review of *Bacteroides* (*Bacteroides*, the good the bad and the nitty gritty) is used as a basic information source for those studying this organism. She has also contributed chapters to prestigious medical textbooks on *Bacteroides*, Anaerobic Bacteria and Metronidazole.

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