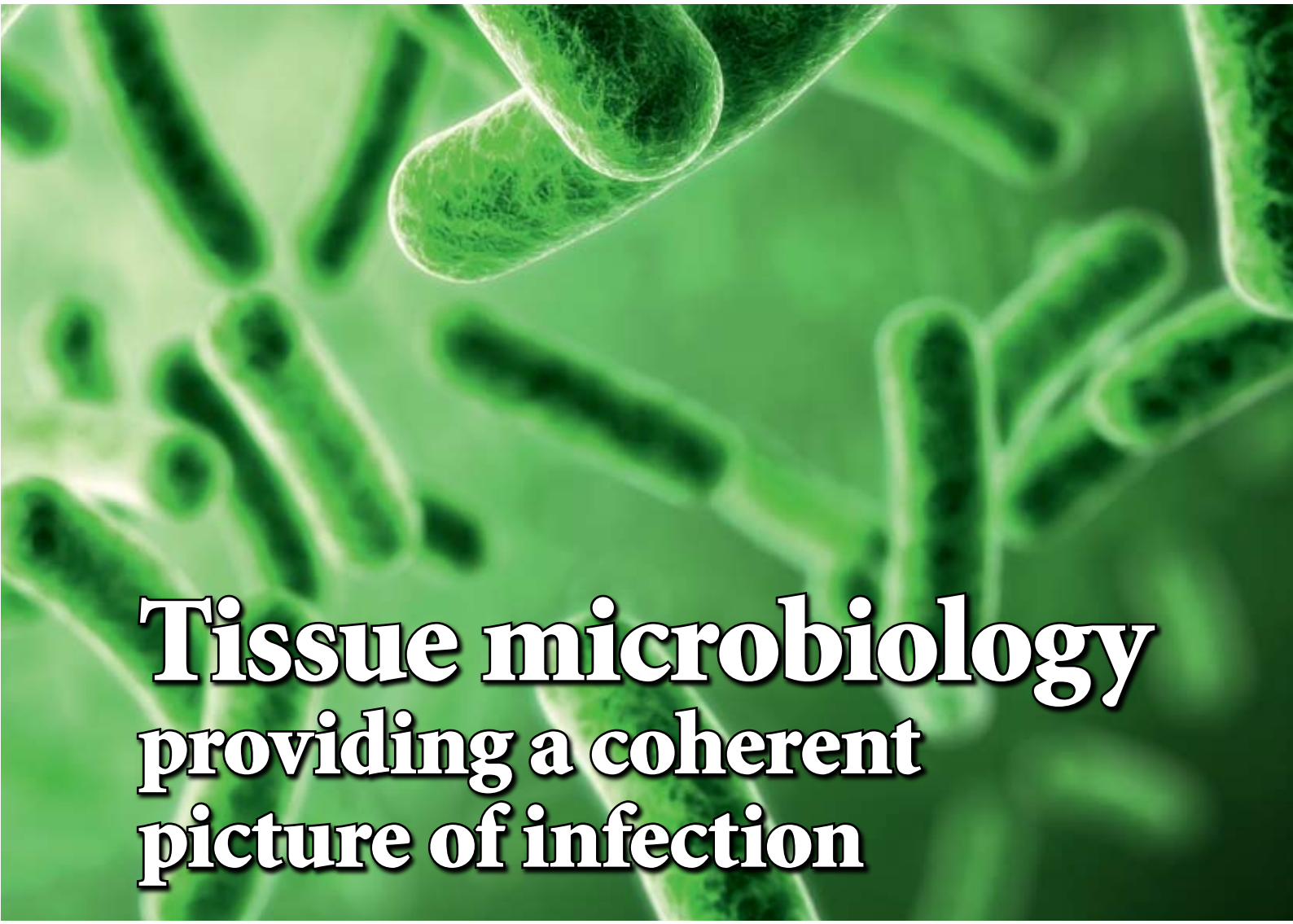
Professor Agneta Richter-Dahlfors and her team at the Karolinska Institute are pioneering a novel research area they term tissue microbiology. They use multiphoton microscopy, molecular techniques, and recordings of physiological functions to study in real-time how bacterial infections impact on tissue homeostasis. The work will likely impact on our understanding of the infectious disease process, and provide targets for novel treatment.



Tissue microbiology providing a coherent picture of infection

Professor Agneta Richter-Dahlfors puts her research in tissue microbiology, into context by simply describing the standard lab procedure for studying bacterial infection. Cells are isolated and grown in a tissue culture dish, to which the bacteria are added; their interactions are monitored over time. While this gives us a good indication about how the individual cells react to bacteria, complex tissue responses, such as those occurring inside the organ of an infected host, remain unclear.

She uses a simple example of real-life infection to illustrate how this technique is sub-optimal for studying the progression of infection within an infected organ. "When you get a splinter in your finger, bacteria contaminating the splinter are introduced into the skin. Numerous signals will then be sent from this site in order to orchestrate the immune system and other tissue events, which collectively are required to clear the infection. That is why it takes a few days before your swollen finger is back to normal."

Using this splinter scenario as a comparison to how the in vitro experiments in the tissue culture dish are done, Richter-Dahlfors neatly illustrates the difficulty to mimic the complexity of tissue events in a dish. This has led to her group pioneering a novel research area looking to visualise in real-time the immediate/early alteration of the tissue homeostasis that accompanies bacterial infections. "Our team has set out to look at what actually happens during the infection process, within an organ of a live animal," she elaborates. "We do so by studying E. coli infections in the rat kidney, since this bacterium is the most common causative agent of pyelopnephrits," she says.

"The holy grail of infection biology is to study a pathogen within its natural environment, the living host. Advances in in vivo imaging techniques have begun to introduce the possibility to visualise, in

real time, infection progression within a living model. With the current advancements and knowledge in multiphoton microscopy, we have together with Professor Bruce Molitoris, director of Indiana Center for Biological Microscopy at Indiana University School of Medicine, developed a new and valuable technique for in vivo imaging of bacterial infections. It has allowed us to study how the microbes act within their natural infectious environment – the living host."

"When the bacteria are introduced into the live kidney, the site can be monitored in the microscope," she continues. "Starting from only a handful of bacteria, we can monitor their rapid multiplication, and how the immune response is activated during this process, which finally leads to eradication of the infection."

There are two perspectives from which to observe the infection process; what the host do in its attempt to kill the bacteria, and how this host response in turn affects the behaviour of bacteria.

"As bacteria were observed to rapidly multiply inside the nephron of the kidney, we noted that the blood flow in the neighbouring vessels stopped. This happens only a few hours into the infection, before the recruitment of immune cells occur. We found that upon bacterial exposure, the renal epithelial cells transmit signals to the cells in the blood vessel, which activated the clotting cascade. As clots are formed in the blood vessel, the blood flow can no longer access the infection site.

"We can measure very accurately the reduction of blood flow, within three to four hours after initiation of bacterial infection. As the blood flow to the infection site ceases, oxygen levels in the tissue drops to zero."

This was a very exciting discovery. When studying tissue next to the infection site, the team discovered that the blood flow was normal.

"That tells us that stoppage of the blood flow is an extremely local event, only occurring at the infection site," confirms Richter-Dahlfors.

At this stage in the research, the team started to ask why the body responds in this way.

"We had no idea that this process would be so rapid," explains Richter-Dahlfors. "Our first surprise was when we noticed that bacteria had been cleared from the site in less than 24 hours. We therefore started to look at the first eight hours as the important time frame for tissue communication and it was surprising to find that oxygen falls to zero within three to four hours."

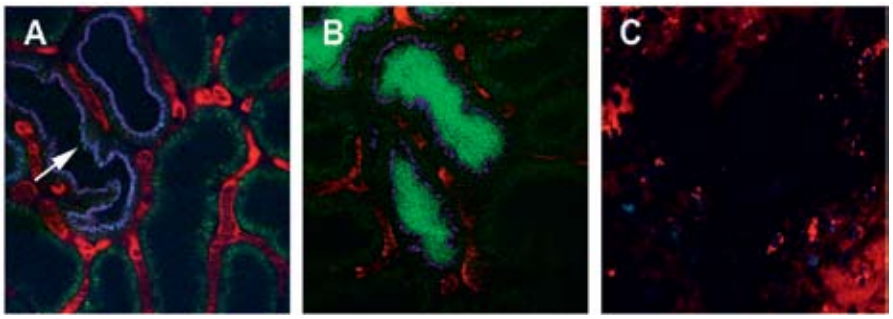
> "If we can use the organ as our test tube, we can start looking at how the microbe actually respond to host defences during the infection process, rather than under ordinary lab conditions. It is about the dynamic interplay between the host and the bacteria"

With this timeframe established, the team then applied clinically used drugs like heparin to stop blood clotting from occurring during infection.

This treatment allowed bacterial spread into the blood stream and the rats died within a few hours due to septic shock.

"This tells us that the clotting occur to allow the host to contain the bacteria at the infection site," says Richter-Dahlfors. "That gives time for immune cells to come into the site and kill the bacteria. From the host perspective, it is a lot better to lose the function of one nephron as the local blood supply is shut off, than to die from sepsis. That is why you try to contain the microbes at the site."

Realizing the major changes of the microenvironment that bacteria must cope



The infection process within the kidney

A In the renal tissue, blood vessels, shown in red, are located very close to the tubules, through which the urine is leading out from the kidney towards the bladder. When bacteria (green) infect the tubule (outlined in blue), they bind to the tubular mucosal cells. These few bacteria (pointed at by the arrow) start multiplying, and within a few hours, a massive green stain, representing thousands of bacteria, is observed inside the tubule

B The alerted immune cells come to the site and eradicate the bacteria (no green stain in panel

C At the same time, their infiltration is destroying the tissue architechture (large, black area).

Images adapted from Månsson et al, Cell Microbiol. 2007, 9(2):413-24.

with during the infection process prompted Richter-Dahlfors and her colleagues to push the term "tissue microbiology".

"Traditionally, we have studied how bacteria behave in test tubes," she reiterates. "During the mid-1980s, researchers started to study how bacteria interacted with eukaryotic cells in culture dishes. This dramatically increased our understanding of the interplay between bacteria and their target cells. From there, we arrived at 'cellular microbiology', the field that describes this interplay – how the bacteria affect the target cells and in turn how the cell affects the bacteria."

Richter-Dahlfors explains how this interplay relates to her research. "If you now think of the nephron inside the kidney as a test tube, bacteria are exposed to a constantly changing environment. While the first bacteria infused into the nephron arrived when there was a normal tissue oxygen level; within a few hours, the oxygen is down to zero.

At this later time point, bacteria thus experience a very different microenvironment, which most likely affects the bacterial gene expression pattern. This is an area that is currently under investigation.

"Bacteria have completely different genes being expressed depending on their growth condition and this is key to 'tissue microbiology'," she concludes. "If we can use the organ as our test tube, we can start looking at how the microbe actually respond to host defences during the infection process, rather than under ordinary lab conditions. It is about the dynamic interplay between the host and the bacteria."

"These kinds of dynamics, where you have changes in nutrient availability, and parameters such as the renal filtrate flow, are not even taken into consideration in the ordinary lab conditions."

However, performing experiments within the live kidney are technically challenging, and this model needs to be balanced by in vitro experiments. Unfortunately, there is a shortage of in vitro systems that properly mimic the in vivo situation, and this has inspired Richter-Dahlfors and her team to interact with engineers in order to improve such experimental platforms.

"As the director of the Swedish Medical Nanoscience Centre at Karolinska Institutet, I am in a fortunate position to closely interact with engineers" she explains. Together, they develop novel devices, aiming to increase their understanding of infectious diseases at a molecular level under conditions that more closely resembles the in vivo situation.

By combining microbial pathogenesis with intravital imaging, innovative surgical procedures, physiological tissue recordings, and molecular analysis, the knowledge about bacteria-induced organ- and tissue specific cell communication will increase. Tissue microbiology will thus become one essential component in the strive to understand the relevance of bacterial virulence factors, and will likely provide a more complete picture of the complex infection process.

At a glance

Project Information

Project Title:
Deciphering the dynamic interactions between bacteria and host tissue during infection

Project Objective:
Bacterial infections are accompanied by dynamic tissue alterations in the infected organ. By visualizing in real-time the immediate/early alteration of tissue homeostasis during infection, and how this subsequently affects bacterial behavior, this project is pioneering the field of "tissue microbiology". Combining microbial pathogenesis, intravital imaging, innovative surgical procedures, physiological tissue recordings, and molecular analysis in the live animal model is essential to provide a more complete picture of the complex infection process.

Project Duration and Timing:
3 years: 2011-01-01-2013

Project Funding:
The Swedish Research Council

Project Partners:
Professor Bruce A Molitoris, Indiana Centre for Biological Microscopy, Department of Medicine, Indiana University School of Medicine, Indianapolis, USA

Agneta Richter-Dahlfors

Richter-Dahlfors is professor in cellular microbiology at Karolinska Institutet. Along with her pioneering work on "tissue microbiology", she is strongly engaged in interdisciplinary research such as integration of organic electronic technologies for biological applications. Richter-Dahlfors holds a key position for interdisciplinary research at Karolinska Institutet as director of the Swedish Medical Nanoscience Center.

Contact

Main contact name:
Professor Agneta Richter-Dahlfors

Tel: +46 (0)8 524 874 25