

Chlamydia (pseudocolored green in this scanning electron microscope image) nest in the interior of human host cells. Up to 1,000 bacteria can be found in one inclusion body.

# The Sabotage of Cell Logistics

The bacterium *Chlamydia trachomatis*, a major cause of infertility and miscarriage in women, as well as blindness in children, is the current focus of **Dagmar Heuer's** research at the **Max Planck Institute for Infection Biology** in Berlin. This ingenious pathogen uses a trick to ensure its survival within its host cell: it exploits the cell's distribution center by diverting the cell's "materials logistics system" for its own use.

TEXT **BARBARA ABRELL**

**C**hlamydia *trachomatis* is a devious pathogen: it is transmitted via, among other things, mucous membranes during unprotected sexual intercourse, and then infiltrates the cells of the uterus and fallopian tubes. Once established there, it cleverly camouflages itself against the defensive attacks of the body's immune system and freeloads in the host cell interior, acquiring the necessary sustenance for survival. Chlamydia unfolds its effect gradually: whereas the bacteria that cause tetanus or cholera flood the body with toxins that disrupt essential cell functions, Chlamydia does not severely harm host cells and tissues directly. Instead, it triggers an excessive defensive reaction. The body's immune system attempts to keep the bacterium at bay through inflammatory responses – but this kind of defensive reaction is precisely what causes the permanent and irreversible damage associated with Chlamydia infection.

Since genital infection initially triggers few or no symptoms, most people do not notice Chlamydia's covert attack, and infections thus often remain untreated. Experts estimate that there are up to 90 million new Chlamydia infections each year. According to the International Union against Sexually Transmitted Infections (IUSTI) – an organization that has been working on

the epidemiological aspects and control of sexually transmitted diseases since 1923 – up to 80 percent of women and 50 percent of men are infected with Chlamydia, and most of them live in developing countries.

## CHLAMYDIA: A HIDDEN EPIDEMIC

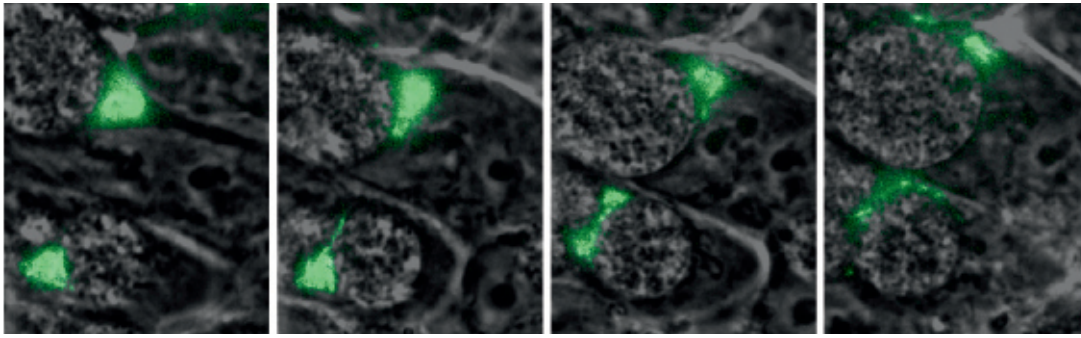
In terms of the number of Chlamydia infections, "there is little reliable data available for Germany, only estimates," says Osamah Hamouda from the Robert Koch Institute in Berlin. An expert on infectious disease, Hamouda believes that frequently published figures to the effect that around 10 percent of all women under 25 in Germany carry the infection are "way too high." "We work on the assumption that between 3 and 6 percent of young women are infected with Chlamydia." Chlamydia infection is the cause of infertility in one third of women who are unable to have children. Moreover, pregnant women infected with the bacterium are far more likely to suffer ectopic pregnancies or give birth prematurely. If the infection is not detected and treated with antibiotics at an early stage, the infected individual can continue to pass it on unwittingly.

Due to their extremely small size and the fact that they are obligate intracellular organisms – meaning they

can reproduce only within their host's cells – Chlamydia were long believed to be viruses. Chlamydiales were first discovered by a German expedition team in Java in 1907. Later scientists discovered that, in addition to humans, these pathogens can also infect other mammals, birds, and even amoebae. With at least 13 different species, Chlamydia are among the most common infectious bacteria.

*Chlamydia trachomatis* is one of only three species that infect humans, and it is Dagmar Heuer's main research interest. The 33-year-old infection biologist, based at the Max Planck Institute for Infection Biology in Berlin, is seeking to pinpoint the pathogen's virulence mechanisms with the help of molecular-biological techniques. Chlamydia has a unique development cycle that is characterized by two distinct stages: the infectious, extracellular stage, when it takes the form of an EB, or elementary body, and is only 0.3 micrometers long (0.3 thousandths of a millimeter) and also does not display any quantifiable metabolic activity. During the intracellular reproductive stage, the organism is known as the RB, or reticulate body; it is around one micrometer long and metabolically active, but not infectious.

Using a light microscope, it is possible to detect whether or not a cell is infected with Chlamydia, as they form



The researchers can track the fragmentation of the Golgi apparatus of cells infected with Chlamydia using GFP.

a protective spherical niche, called an inclusion, in which they survive and replicate. During the course of an infection, the niche expands and can ultimately accommodate up to 1,000 bacteria. Just 24 hours after infection, the inclusions can be almost as large as the nucleus of the host cell. "It is amazing how quickly these inclusions form and how much space they can take up in the host cell without killing it," explains Dagmar Heuer.

At the institute, Chlamydia is cultivated within HeLa cells at 35 degrees Celsius in Biosafety Level 2 laboratories. These cells were first discovered during the removal of a cervical carcinoma (cervical cancer tumor) from the patient Henrietta Lacks (hence the acronym HeLa) at the Johns Hopkins Hospital, USA, in 1951. While testing the cells for malignancy, doctors observed that some epithelial cells from the carcinoma propagated easily. Since this discovery, HeLa cells have been widely used in research.

The relationship between the bacterial pathogen *Chlamydia trachomatis* and its human host cell can be studied very successfully with the help of these cell cultures. Dagmar Heuer is particularly interested in how this particular pathogen obtains nutrients while ensconced within host cells. Since obligate intracellular bacteria have only limited metabolic capacities, these pathogens must acquire important metabolic products – including amino acids and, in particular, fats – from their host cells. How they do this is revealed by coupling microscopy and digital pho-

tography of individual cells. Heuer's colleague, Volker Brinkmann, combines these images to make a 'film' that clearly reveals the pathogen's protective niche within its host cell. Cell membranes can be clearly differentiated from the cell cytoplasm under the light microscope, as they scatter light differently. "The Golgi apparatus can be seen in fluorescent green," explains Brinkmann. The researchers marked the cell's transport and distribution center with GFP, green fluorescent protein, a protein marker that originates from jellyfish. GFP has become a standard and highly successful tool in the army of cellular biology: Osamu Shimomura, Martin Chalfie and Roger Y. Tsien were awarded the Nobel Prize for Chemistry in 2008 for its discovery and development.

### DIVERTING THE CELLULAR MATERIALS FLOW SYSTEM

"The Chlamydia succeed in exploiting this cellular distribution center for their own purposes, and in diverting the cell's 'materials logistics'," says Dagmar Heuer. The Golgi apparatus consists of a stack of flat membrane-bound cisternae. One side of this cell organelle – the entry side, so to speak – faces the endoplasmic reticulum (ER); the other side – the exit side – faces the plasma membrane. The ER permanently ties off small membrane-bound bubbles, known as vesicles, which are filled with proteins and lipids. These are absorbed by the Golgi apparatus and then migrate through the membrane stack,

also undergoing various changes as part of this process. They then leave the Golgi system via vesicles at the side facing the plasma membrane, and are directed – now bearing the corresponding "address labels" (so-called targeting proteins on the surface of the vesicle) – to specific locations in the cell, or are transported out of the cell.

In cells infected with Chlamydia, the Golgi apparatus fragments into smaller units that, as identified with the help of GFP, line up along the inclusion body. "The small lipid vesicles tied off by the Golgi apparatus are taken up into the inclusion bodies and then incorporated by the Chlamydia into their own membranes," says Heuer. The scientists were able to demonstrate that the bacteria enzymatically splits an important protein – Golgin-84 – located on the surface of the Golgi apparatus. "If you block the cleavage of Golgin-84, the Golgi apparatus does not fragment," explains Heuer. "The bacteria cannot acquire the necessary lipids and mature, so the transformation into the infectious elementary bodies is impeded." The development cycle thus aborts.

Conversely, Heuer and her colleagues Anette Rejman Lipinski, Alexander Karlas and Nikolaus Machuy can trigger the breakdown of the Golgi apparatus by muting the gene that codes for Golgin-84. Chlamydia reproduced rapidly in cells that were unable to produce Golgin-84, and these infected cells released significantly more infectious particles into the environment. This, in turn, infected many healthy

cells. The researchers used the method of RNA interference to “silence” the function of the Golgin gene within the cell. Short strands of RNA – usually less than 30 base pairs – are introduced into the cell and bind to identical sequences within the targeted gene. The pairing of these two individual RNA strands results in a functionless double strand. As a result, the ribosomes – the protein production factories of cells – lack their instructions, and the protein can no longer be produced.

### A PROMISING APPROACH FOR NEW TREATMENTS

This study represents an important milestone in the study of the infection process of *Chlamydia trachomatis*, stresses Thomas F. Meyer, an infection biologist and Director of the Department of Molecular Biology at the Berlin-based Max Planck Institute. This fact is clearly confirmed by its publication in the prestigious scientific journal NATURE. It is Meyer’s hope that the Max Planck researchers “will soon succeed in targeting and starving the pathogens in this way.”

Heuer and her team have already managed to identify two factors that prevent the fragmentation of the Golgi apparatus: two different enzyme blockers called protease inhibitors. These hinder the fragmentation of the Golgi apparatus triggered by Chlamydia and thus prevent the bacteria from obtaining the lipids that are so important for their survival. The two inhibitors identified have now been patented for possible application against

Chlamydia. The research team is currently testing their function in animal models with the aim of developing these substances into novel drugs.

The present challenge is the discovery of other factors that influence Chlamydia infection. The Berlin-based scientists have already identified 1,500 human genes as part of a screening process. “We were able to work with 50

of them,” says Machuy. The team will now delve further into their function in the coming years. This is no easy undertaking, as Chlamydia not only cleverly eludes the body’s immune system, but it is also highly resistant to laboratory investigation. Due to its obligate intracellular lifestyle, it has not yet been possible to genetically manipulate this pathogen. ◀



above: The research team at the Max Planck Institute for Infection Biology in Berlin (from left to right): Volker Brinkmann, Dagmar Heuer, Annette Rejman Lipinski and Nikolaus Machuy.  
below: The researchers grow the bacteria in HeLa cells. Dagmar Heuer examines the cell cultures under the microscope.