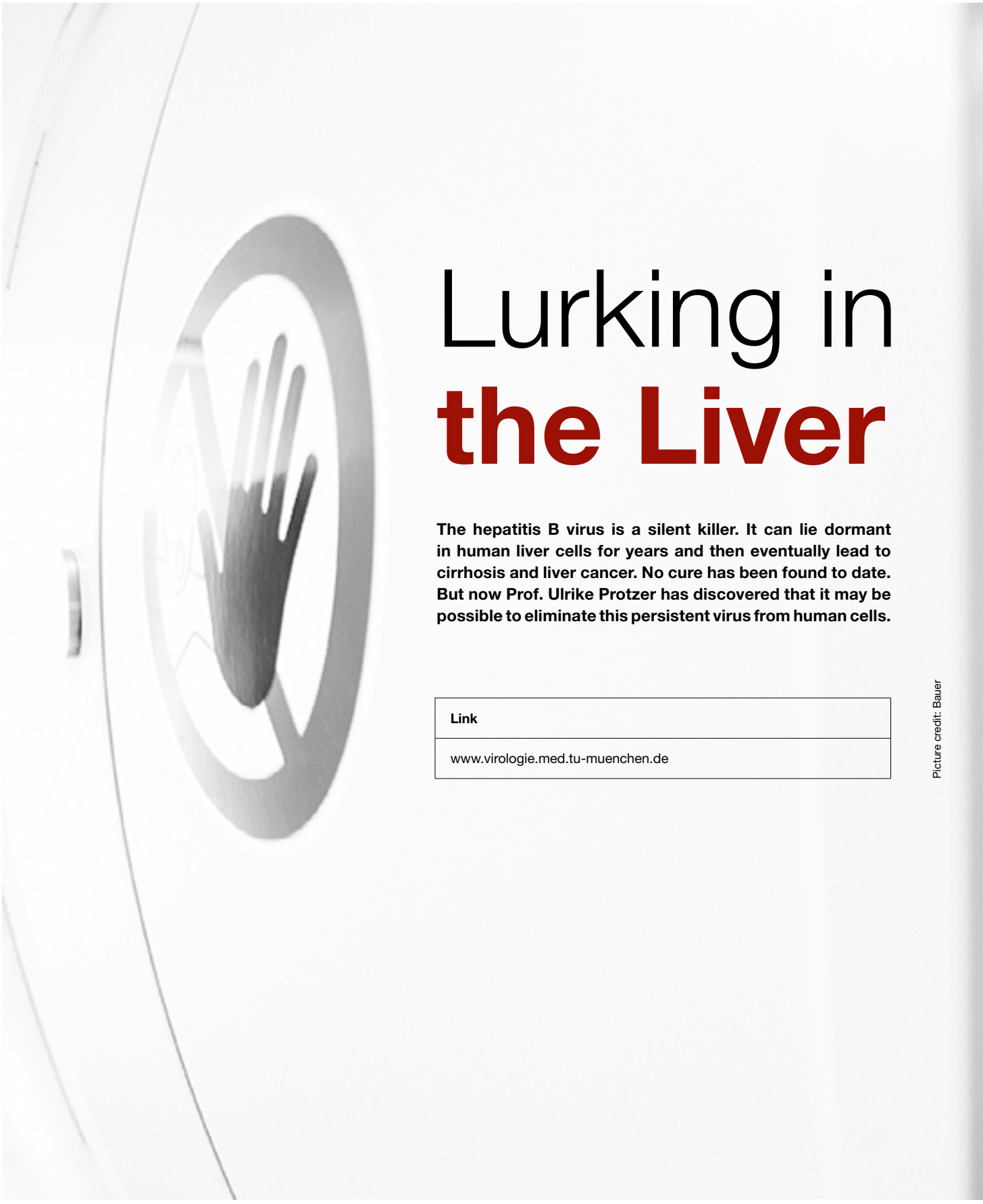
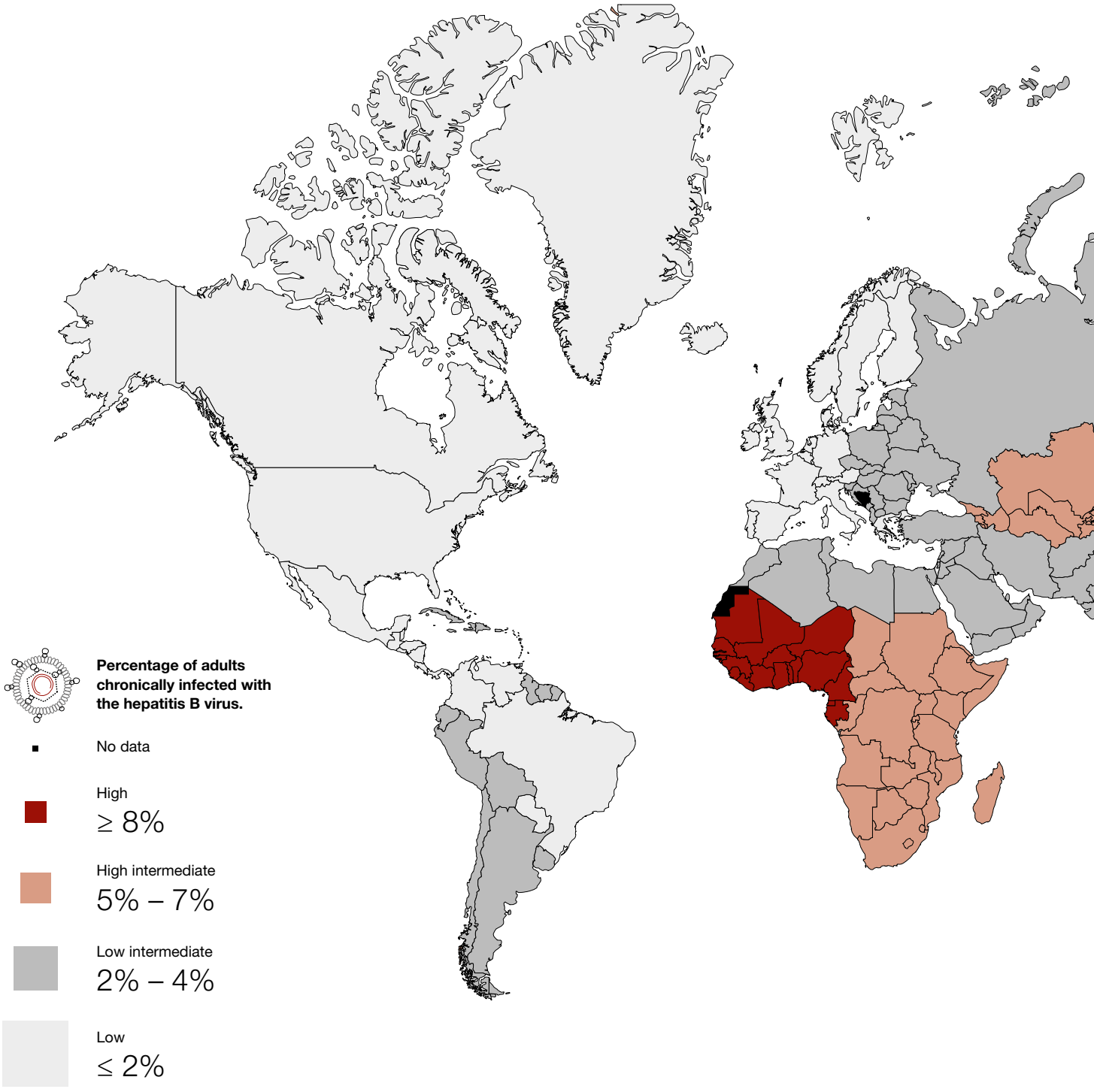


Lurking in **the Liver**

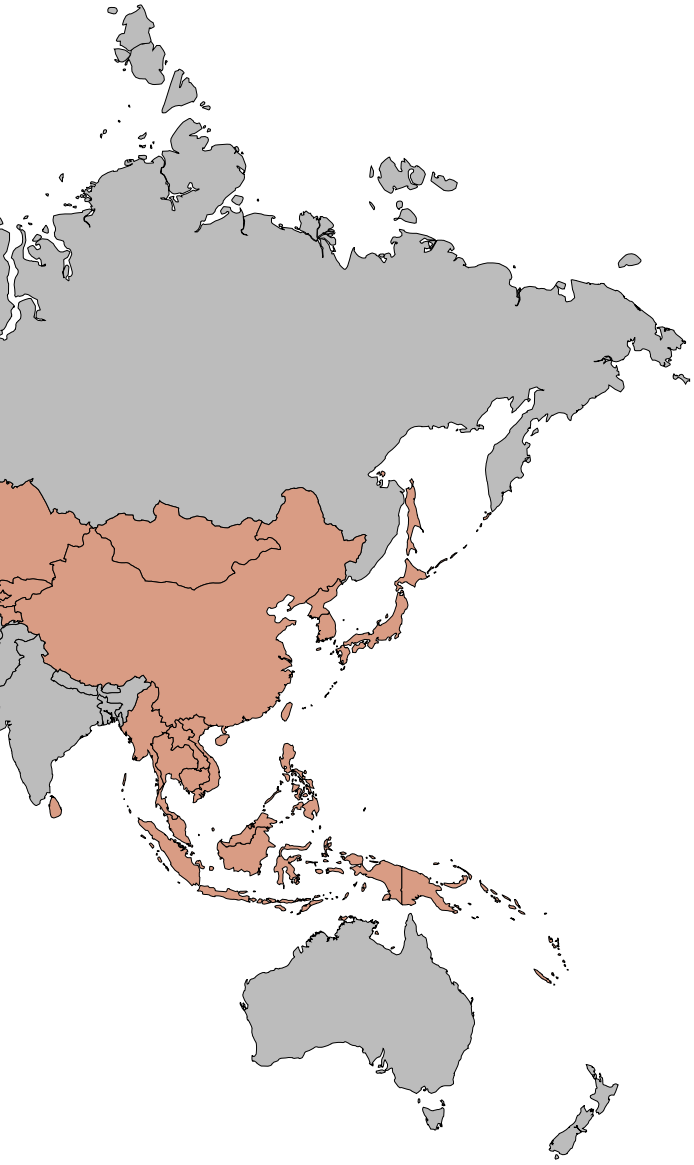
The hepatitis B virus is a silent killer. It can lie dormant in human liver cells for years and then eventually lead to cirrhosis and liver cancer. No cure has been found to date. But now Prof. Ulrike Protzer has discovered that it may be possible to eliminate this persistent virus from human cells.

Link
www.virologie.med.tu-muenchen.de

Picture credit: Bauer



Geographic distribution of chronic hepatitis B virus infection. With about 250 million carriers, hepatitis B virus infection is highly prevalent around the globe. This map shows the prevalence of hepatitis B surface antigen (HBsAg) as a marker of chronic HBV infection among adults in different countries.



Picture credit: ediundsepp (source: TUM)

Gefährliches Leben in der Leber

Das Hepatitis-B-Virus tötet aus dem Hinterhalt. Es befällt zunächst unbemerkt menschliche Leberzellen und führt Jahre später zu Leberzirrhose und Leberkrebs. Hepatitis B wird meist bei oder direkt nach der Geburt übertragen. Bei Säuglingen und Kleinkindern schafft das Virus es in 90 Prozent der Fälle, seine DNA dauerhaft in den Zellkernen der Leberzellen zu hinterlassen. Schätzungen gehen davon aus, dass insgesamt 250 Millionen Menschen dauerhaft mit dem Hepatitis-B-Virus infiziert sind. Eine Heilung gibt es bisher nicht. Doch die Virologin Prof. Ulrike Protzer hat gleich zwei Möglichkeiten entdeckt, das persistente Virus aus den Zellen zu verbannen.

Zerstörung der Virus-DNA

Jahrelang dachte man, dass nur eine Zerstörung der infizierten Leberzellen die Chance auf Heilung bringt. Ulrike Protzer hat herausgefunden, dass es auch anders geht. Stimuliert man spezielle Rezeptoren auf den Leberzellen, so stellen die Zellen sogenannte APOBEC-Proteine her. Diese greifen gezielt die Virus-DNA an, schneiden einzelne Basen kaputt und führen somit zum Abbau des viralen Erbguts. Die Methode ist faszinierend, hat jedoch einen Haken. Nur 40 bis 60 Prozent der Virus-DNA können auf diesem Weg vernichtet werden. Um das Virus ganz zu vertreiben, braucht man die Hilfe des Immunsystems.

T-Zell-Therapie

Die T-Zellen sind dafür verantwortlich, infizierte Körperzellen zu zerstören. Bei Patienten mit chronischer Hepatitis B attackieren aber die T-Zellen die infizierten Körperzellen nicht. Deshalb helfen die Forscher nach, indem sie T-Zellen mit speziellen Rezeptoren versehen, die das Oberflächenprotein des Hepatitis-B-Virus als gefährlich erkennen und sowohl infizierten Leberzellen als auch Leberkrebszellen zerstören. Für eine Therapie wäre es am besten, beide Methoden zu kombinieren.

Claudia Steinert

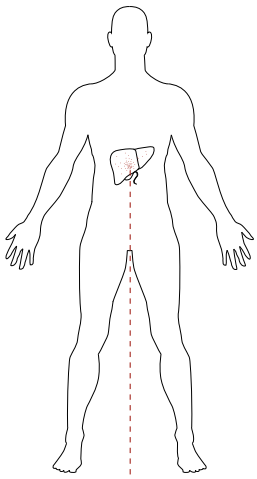
Anyone who has exchanged e-mails with Ulrike Protzer will have a fair idea of the long hours she works. She usually responds in the morning around seven or in the evening after the late-night news. In between, the professor of virology at TUM and Director of the Institute for Virology at Helmholtz Zentrum München is busy trying to develop a cure for chronic hepatitis B.

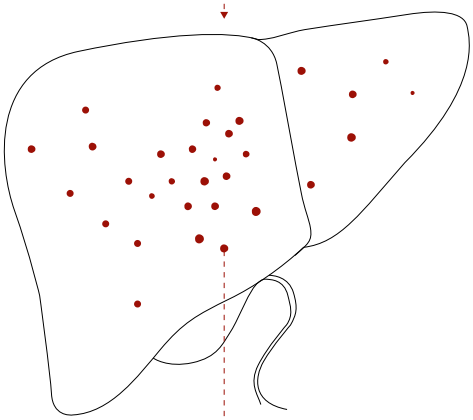
It is estimated that over 250 million people suffer from persistent hepatitis B virus infections. By way of comparison, the number of people infected with HIV currently stands at just 35 million. Hepatitis B is one of the world's most prevalent infectious diseases, responsible for around 500,000 deaths every year. This disease takes a particularly strong toll in Eastern Europe, Africa and Asia.

Ulrike Protzer knows all this from first-hand experience. She has worked in Africa and had to watch young people succumb to a virus that had been "hiding" in their liver for a long time. Even though most infections occur at the time of birth or shortly afterwards, there are no symptoms in the early stages. The virus keeps a low profile – it wants to be passed on to the next generation, so it needs its ▷

Picture credits: Bauer

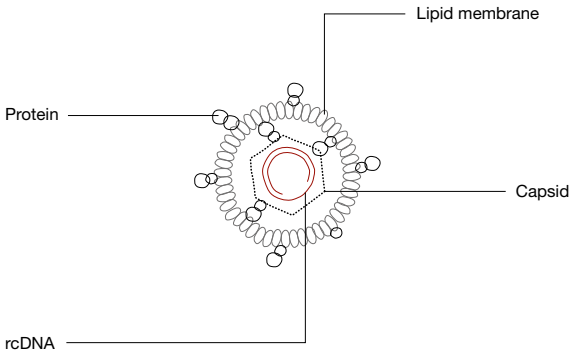
Diagnostics of virus infections. To allow correct diagnosis of viral infection, quality-assured modern diagnostics are essential. Patient material from the clinics is initially processed and prepared (top left) for analysis with standardized diagnostic assays (bottom left). Before reports are finalized, specialized medical virologists discuss the results (right).

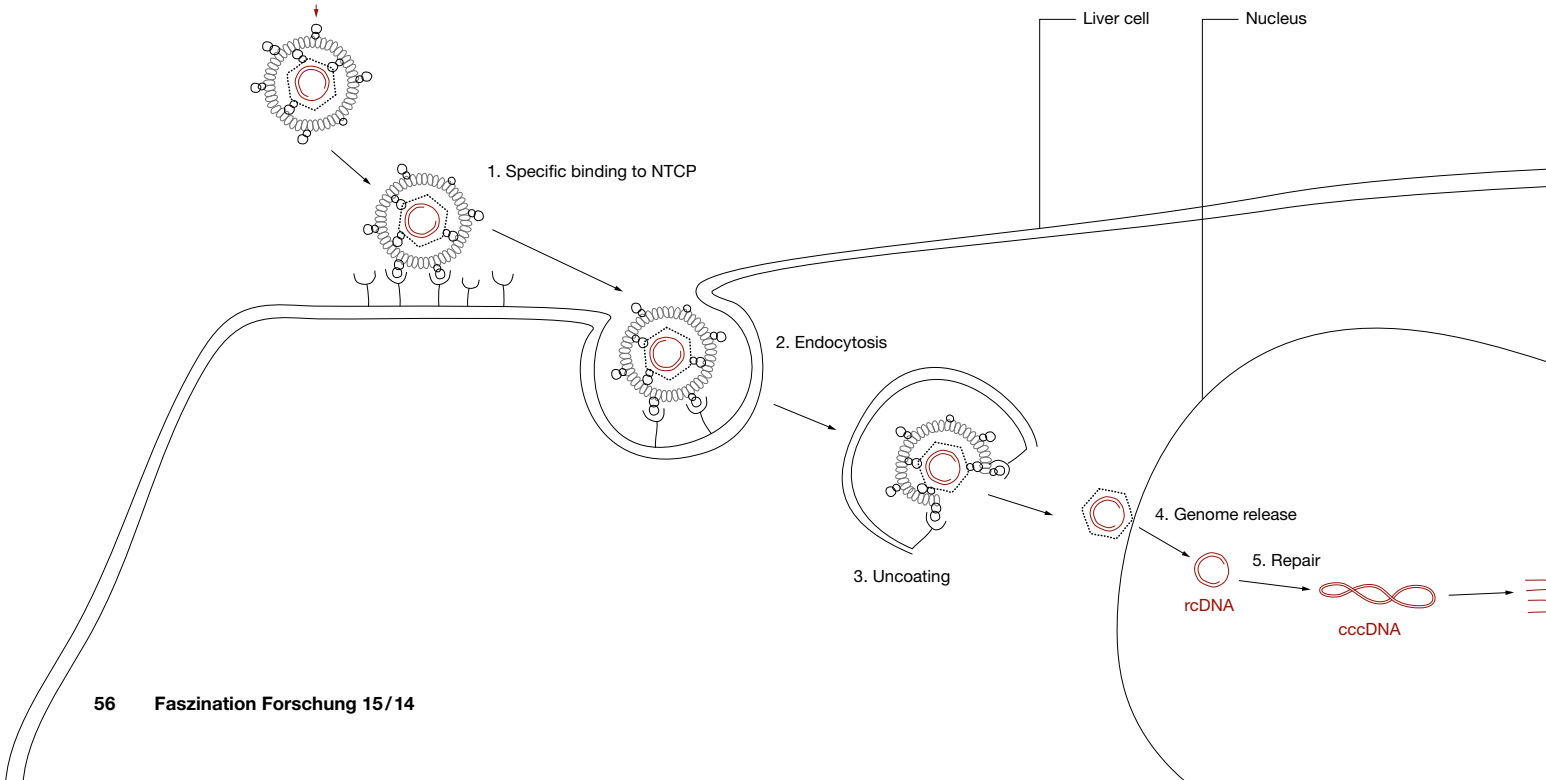




A virus with unusual DNA

The hepatitis B virus is extremely host-specific – it can infect only humans and chimpanzees. It is characterized by its circular DNA, which is only partially double-stranded – one strand is shorter than the other. Only when this strand is completed in the liver cells does the virus assume its permanent form: covalent closed circular DNA (cccDNA), which persists as a minichromosome in the cell nucleus.





human host to remain healthy and capable of reproduction for as long as possible.

It is only when the infected person has become a young adult and passed the virus on to their own offspring that the damage caused to the liver becomes clear. Cirrhosis and liver cancer (known as hepatocellular carcinoma) are the most common long-term consequences. These conditions are untreatable in most cases, with 50 percent of patients dying within six months of diagnosis.

The virus is engineered to pass from mother to child. While the immune system of adults is well equipped to fight the hepatitis B virus, children can't efficiently eliminate the virus. In fact, 90 percent of all babies and small children who come into contact with the virus become chronic virus carriers. The figure for adolescents and adults is just 5-10 percent.

This complicates efforts to prevent infection in the first place. There is a vaccine, but it has to be administered prior to infection or – to stop the virus being passed on from mother to child – in the first 24 hours after birth. In rural China or remote African villages, this is simply not practical. "Wiping out this disease through vaccination alone will be very, very difficult," stresses Protzer. That is why her research is concentrating on ways to eradicate the virus from the liver cells. While the latest drugs are capable of controlling hepatitis B, they cannot eliminate it.

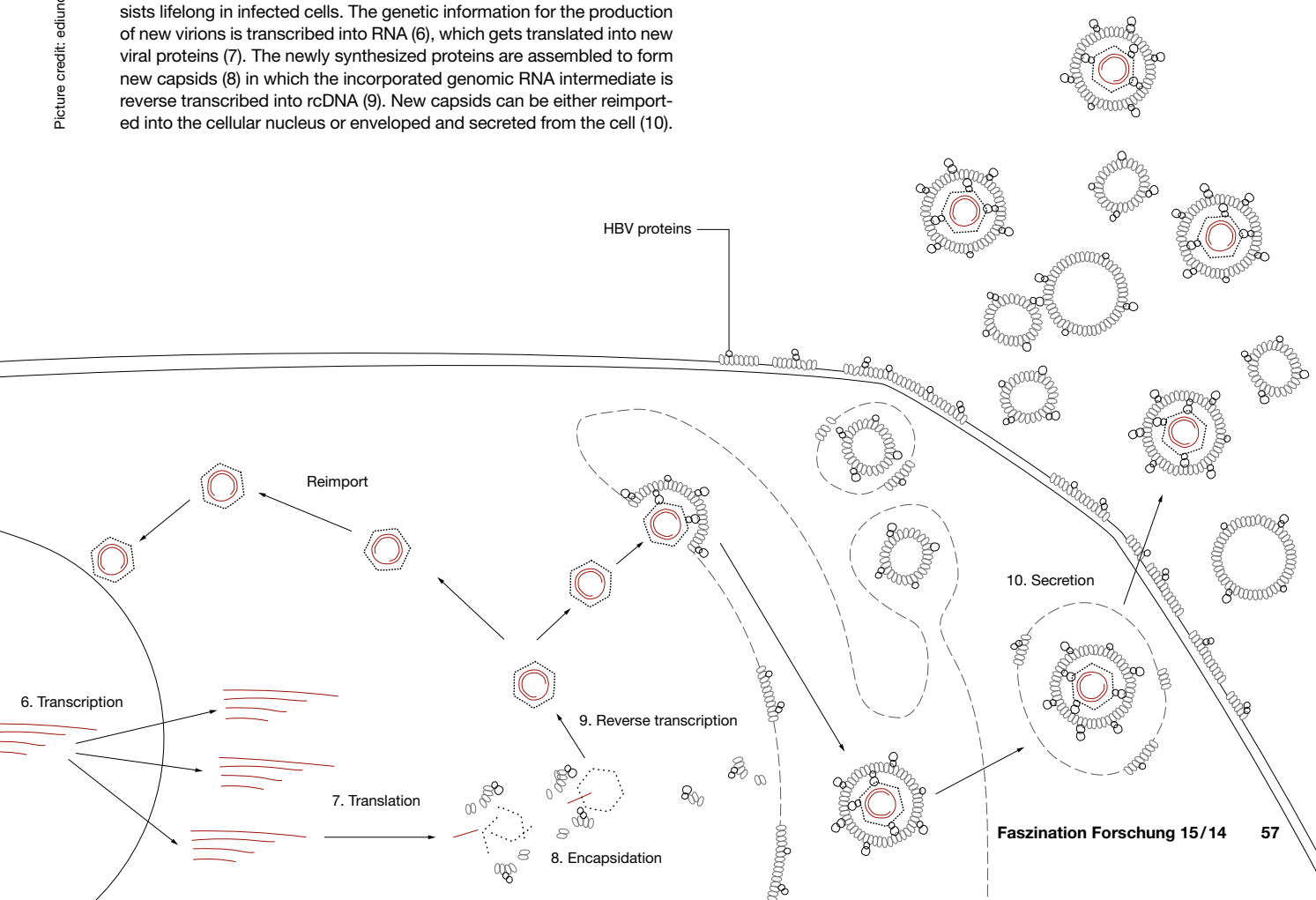
The virus uses the liver cells

The hepatitis B virus is amazingly small and unbelievably efficient. Its genome consists of just 3,200 base pairs containing all of four genes. "That this thing can survive at all is astonishing in itself," says Protzer. The human genotype is a million times bigger, and even the herpes viruses have 50 to 70 times more DNA than hepatitis B.

When a person becomes infected, the virus circulates in their blood until it reaches its target: the hepatocytes in the liver. A transporter with the job of conveying bile acid from the blood to the liver "smuggles" the hepatitis B virus into the cells unnoticed. The virus then latches on to the cell nucleus, opens its capsid and imports its tiny genome ▷

Picture credit: ediundsepp (source: TUM)

The hepatitis B virus uses liver cells (hepatocytes) for reproduction. The free virus (virion) recognizes hepatocytes via the NTCP receptor (1) and is taken up by invagination of the cell membrane (2). The virus is released into the cytoplasm (3) and its genome (rcDNA) is imported into the nucleus (4), where it is converted (5) into so-called cccDNA that persists lifelong in infected cells. The genetic information for the production of new virions is transcribed into RNA (6), which gets translated into new viral proteins (7). The newly synthesized proteins are assembled to form new capsids (8) in which the incorporated genomic RNA intermediate is reverse transcribed into rcDNA (9). New capsids can be either reimported into the cellular nucleus or enveloped and secreted from the cell (10).



Picture credit: Bauer

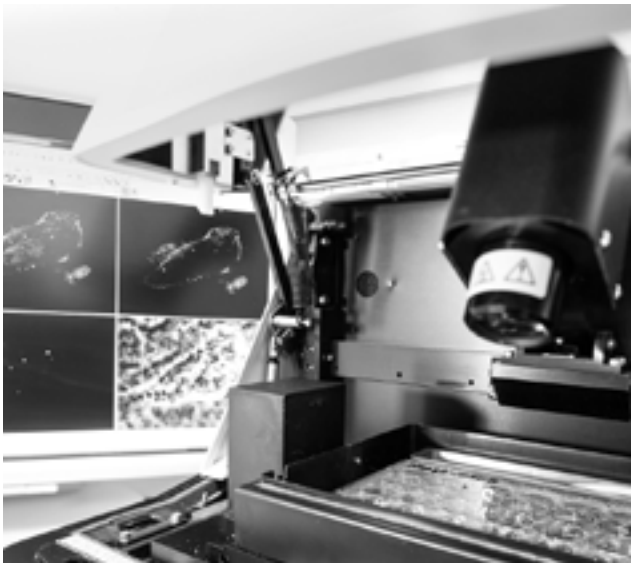
into the cell nucleus via a mechanism that is not yet fully understood. The cell is an active assistant in this process. "We are not sure how the virus DNA gets into the nucleus through the nuclear pores, but we do know that cellular proteins are involved," affirms Protzer.

Once the virus has penetrated the cell nucleus, the cell does all of the work for the virus. First, it assists in converting the only partially double-stranded, circular virus DNA into its permanent form cccDNA. It does this by inserting the missing base pairs and fixing the loose ends. Only then can the virus genes be transcribed and proteins produced. Here too, the human cell lends a helping hand. Protzer explains the dilemma: "If we tried to disrupt the viral protein synthesis or the DNA transcription, we would end up damaging the cellular enzymes. Hepatitis B ensures that we have very few areas to target."

Very few weak points

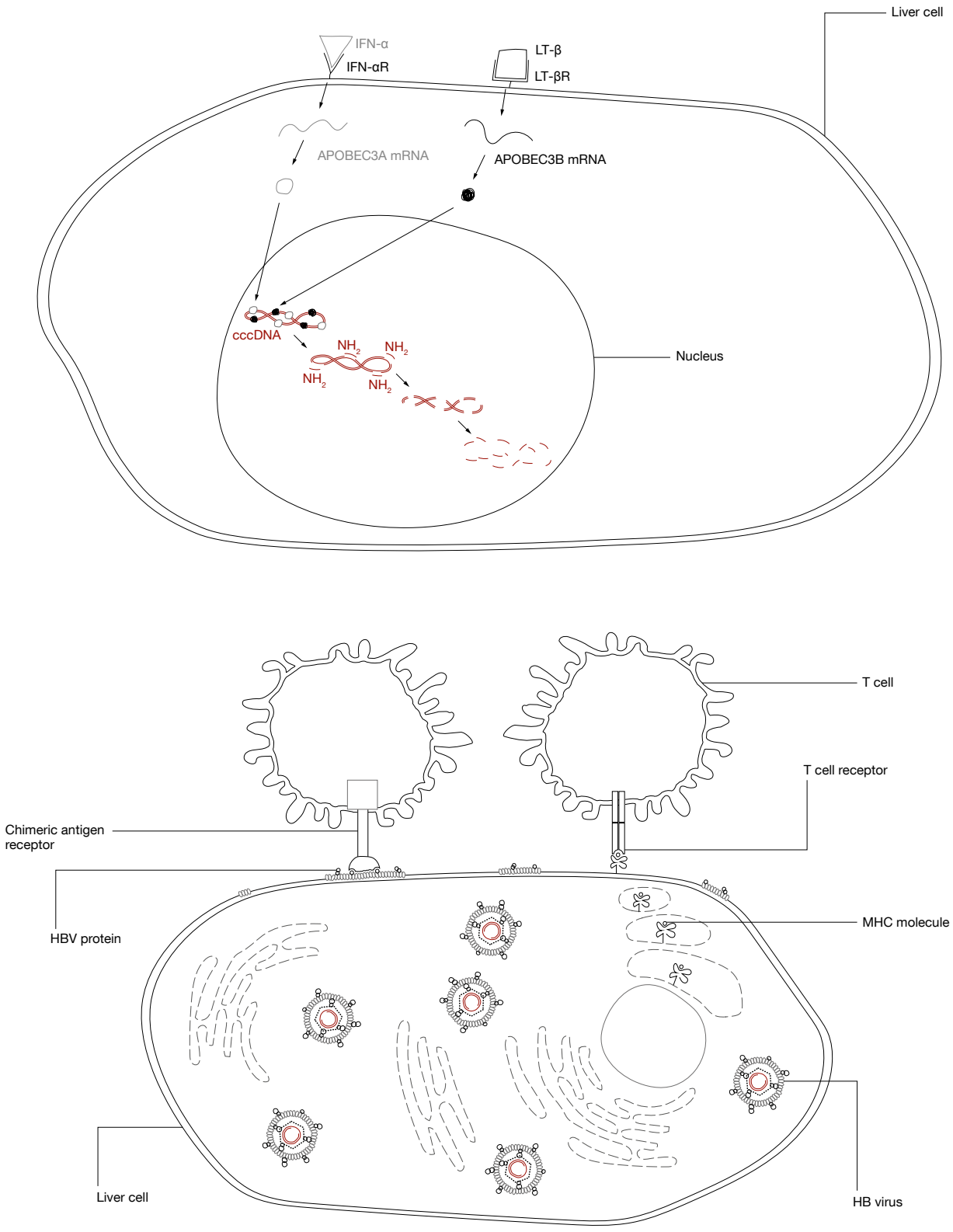
Recently, Ulrike Protzer succeeded in finding one of these rare weak points to target. Together with her research team, she discovered a way to degrade the cccDNA in the cells without damaging the liver cells. "It was fascinating to see that there are actually ways for the immune system to eliminate a particular DNA from the cell nucleus, because that can easily become critical for the cell," she relates. The researchers used special immune mediators to stimulate receptors on the surface of the liver cells. Signal pathways are then triggered inside the cell, and these result in the formation of APOBEC proteins. These proteins belong to the large group of deaminases. They function rather like scissors, specializing in cutting off amino groups (nitrogen-containing side chains) from other molecules.

The APOBEC proteins bind to a virus protein and are thus conveyed to the cccDNA. There, they proceed to cut up one of the building blocks of DNA, the base cytosine. Dozens of amino groups detach the APOBEC proteins, making the virus DNA useless. Eventually, it is completely degraded. In theory, the cell could repair the faulty virus DNA, but luckily, it does not. "It could be that the cell simply capitulates in the face of too many faults," reckons Protzer. The fact ▷



Picture credits left: Bauer, right: ediundsepp (source: TUM)

State-of-the-art technology enables sophisticated bench work and translation of basic findings into clinical applications. Confocal microscopy with an incorporated incubation chamber allows real-time imaging of virus infection and reconstruction of three-dimensional images of infected cells (left). Generation and cultivation of genetically modified T cells is being adapted to "good manufacturing process" guidelines (a regulatory requirement for clinical use) to enable a translation of this approach into clinical applications (right).



Different approaches to eliminate HBV-infected hepatocytes. Top: Upon treatment with cytokines like IFN-α or LT-β, infected cells upregulate APOBEC deaminases. These proteins enter the nucleus and remove amine residues from the viral cccDNA, finally resulting in fragmentation of the viral genome. **Bottom:** Infected cells can be recognized by T cells engrafted with genetically modified receptors. These are optimized either for recognition of HBV proteins on the MHC molecules produced by the cell (right) or for binding to HBV proteins incorporated into cellular membranes (left).

Working for twenty years in infectious diseases and infection research, Prof. Protzer has specialized in viral hepatitis. With recent advances in the field, including her own findings about novel ways to eliminate the virus from infected cells, she is driven more than ever by the goal to cure patients with hepatitis B.

Picture credit left: Bauer, right: Thomas Bock/Hanswalter Zentgraf, German Cancer Research Center

"I would love to see some improvement here, because there will always be some new infectious diseases to combat, whether it be flu or Ebola."

Ulrike Protzer

that Protzer and her team have found a mechanism to eliminate the virus DNA from liver cells without destroying them opens up exciting new therapeutic possibilities. For a long time, researchers thought that chronic hepatitis B could be treated only by completely destroying the infected cells.

There is one catch, however. In experiments carried out so far, it has never been possible to eliminate all of the cccDNA. The researchers were able to generally degrade 50 to 60 percent, or at most 80 percent, of the virus DNA in this way. "For the rest," says Ulrike Protzer, "we need the help of the immune system."

T cell therapy to destroy infected or abnormal cells

To be more exact, help is required from the T cells. As part of the immune response, T cells destroy infected or abnormal cells in the body. The diseased cells draw attention to themselves by presenting fragments of viral proteins, known as antigens, on their surface, thereby attracting T cells.

The fact that 90 percent of all adults who come into contact with the hepatitis B virus are able to clear the infection with their immune system shows that the body has its own defense mechanism. But in patients who are chronically infected, the immune system is not up to the task. Like a bloodhound unable to pick up the scent because of a cold, the killer cells aimlessly pass by the infected hepatocytes instead of attacking them.

"That is why we have to activate the T cells and specifically direct them toward the infected cells," explains Protzer. This approach has already worked in a mouse model. The scientists took blood from infected mice and added new receptors to the T cells. These chimeric antigen receptors were modified to recognize the surface protein of the hepatitis B virus and classify it as foreign and dangerous.

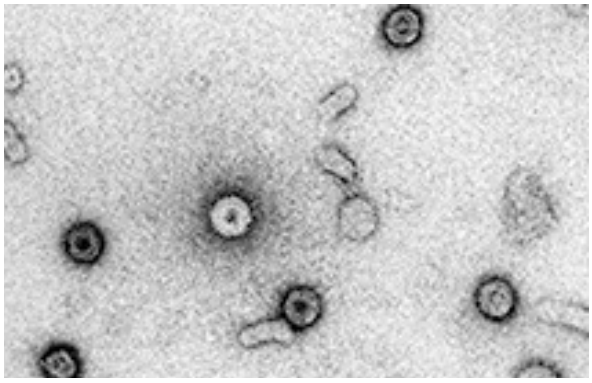
When injected back into the mouse, the modified T cells with their new receptors become immediately aware of diseased cells in the liver tissue. They latch on to them and release cytokines. With these semiochemicals, they prompt neighboring cells to search for virus DNA in their cell nuclei, too. Finally, the T cells destroy the infected somatic cells. Since both chronically infected cells and liver cancer cells display the surface protein of hepatitis B, infected and abnormal or cancerous cells can be destroyed with the same mechanism. This T cell therapy has made headlines in recent years in connection with the treatment of tumors in particular. Indeed, the journal "Science" declared it the breakthrough of the year in 2013. "I think that we will have to combine both methods to arrive at a treatment for chronic hepatitis B: destroying the cccDNA with the APOBEC proteins in conjunction with T cell therapy. We are currently investigating whether we can replace the modification of the T cells with specific antibodies, thus simplifying the therapy," relates Protzer. Her mission in life is to develop a treatment for chronic hepatitis B. Having now discovered more than one mechanism to eliminate the virus from the liver, she is a few steps closer to realizing her dream.

Ulrike Protzer hopes to see more medical professionals choosing the research avenue to search for treatments for dangerous infectious diseases. Even though Germany has an excellent basic research platform for hepatitis B, there is still a shortage of people who are able to bridge the gap between laboratory and clinical practice. "I would love to see some improvement here, because there will always be some new infectious diseases to combat, whether it be flu or Ebola," she concludes. And she already has her hands full with hepatitis B!

Claudia Steinert

History

In 1964, physician Baruch Samuel "Barry" Blumberg found a new antigen in the blood of Australian aborigines. He named it the Australia Antigen. A few years later, the related hepatitis B virus was discovered. This virus causes acute and chronic liver infections and also plays a major role in the development of liver cancer. A hepatitis B vaccine was created in 1982, and it could be called the world's first anti-cancer vaccine. In 1976, Blumberg and Daniel Carleton Gajdusek jointly received the Nobel Prize in Physiology for their discovery.



Electron microscope image of hepatitis B viruses