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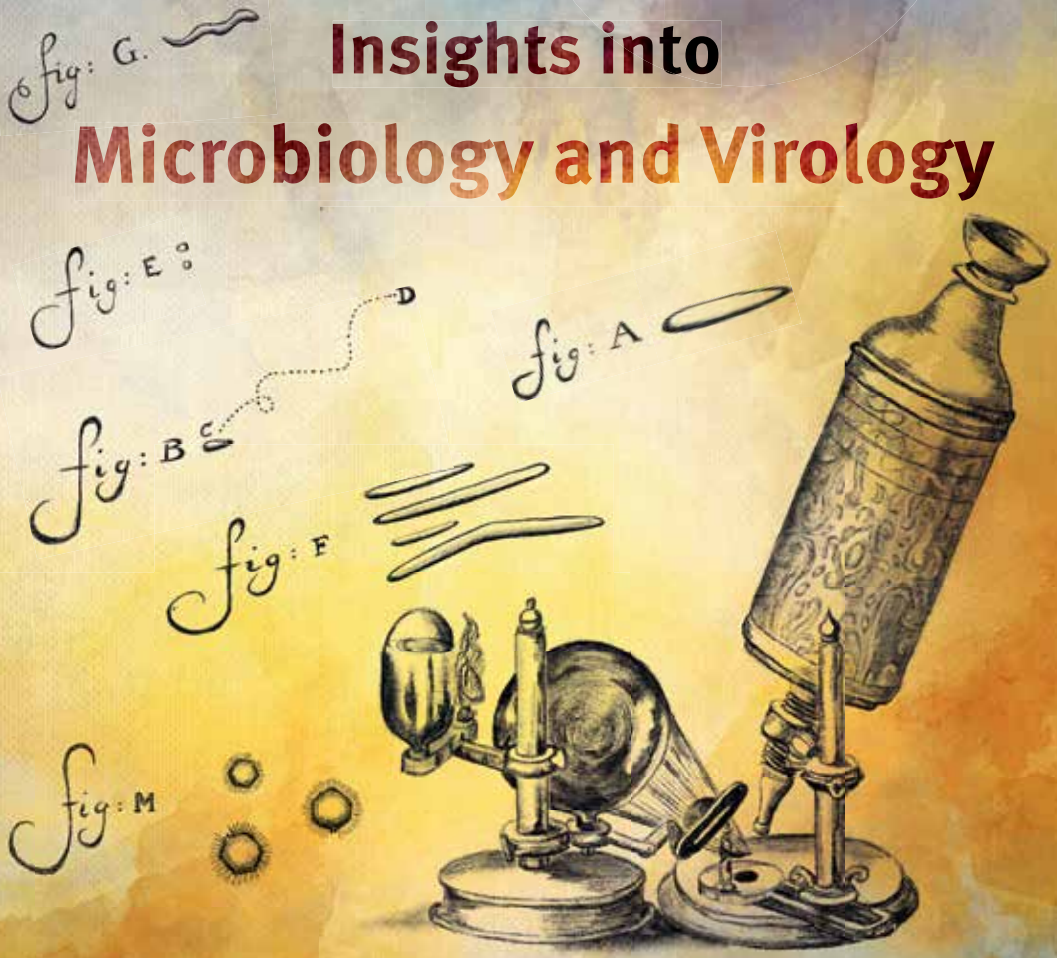
Get Infected!

Insights into Microbiology and Virology



12th Student Symposium on Molecular Medicine

Morning Session

09:00- 9:10	**Michael Kühl** *Dean of Studies of Molecular Medicine, Ulm University*	**Welcome Address**
09:10- 09:55	**Daniel Sauter** *Institute of Molecular Virology, Ulm University Medical Center*	Host vs. virus: how a million-year arms race shaped our immune system
	Maximilian Große *Institute of Molecular Virology, Ulm University Medical Center*	Guanylate-binding proteins 2 and 5 inhibit viral replication by targeting the maturation of viral envelope proteins
09:55- 10:40	**Ralf Möller** *German Aerospace Center (DLR), Institute of Aerospace Medicine, Cologne*	"To boldly go where no microbe has gone before" – fascination and responsibility of space microbiology
	Katharina Siems *German Aerospace Center (DLR), Institute of Aerospace Medicine, Cologne*	Microbial biofilms in space – Evaluating copper-based antimicrobial surfaces in the upcoming ESA space experiment BIOFILMS
10:40- 11:05	**Coffee Break**	
11:05- 11:50	**Friedrich Frischknecht** *Department of Infectious Diseases, Parasitology, Heidelberg University*	Cell biology of malaria parasites in the mosquito
	Manuela Aguirre Botero *Department of Infectious Diseases, Parasitology, Heidelberg University*	Analysis of α-tubulin isotypic differences in Plasmodium berghei microgamete formation
11:50- 12:35	**Stefan Taube** *Institute for Virology and Cell Biology, University of Lübeck* **Marit Lingemann** *Institute for Virology and Cell Biology, University of Lübeck*	Development of an optimized DNA and RNA-based murine norovirus reverse genetics system
12:35- 14:00	**Lunch Break incl. Poster Session**	

Afternoon Session

13:15- 14:00	**Poster Session**	
14:00- 14:30	**Thomas Mertens** *Institute of Virology, Ulm University Medical Center/ Robert Koch Institute (STIKO), Berlin*	Vaccination, Vaccines and Vaccination Recommendations
14:30- 15:15	**Daniel Unterweger** *Max Planck Institute for Evolutionary Biology, Plön*	Bacteria interact with surrounding cells... at war with each other
	Marie Vallier *Max Planck Institute for Evolutionary Biology, Plön*	Bacteria interact with surrounding cells... as evolutionary driver of the host
15:15- 15:25	**Poster Prize Award Ceremony**	
15:25- 16:05	**Coffee Break**	
16:05- 16:50	**Norbert Tautz** *Institute of Virology and Cell Biology, University of Lübeck*	Structural basis for the switch between RNA replication and virion morphogenesis in pestiviruses
	Thomas Walther *Institute of Virology and Cell Biology, University of Lübeck*	Determination of the membrane topology of non-structural protein 2 (NS2) of pestiviruses
16:50- 17:00	**Closing Remarks**	

Abstracts

Host vs. virus: how a million-year arms race shaped our immune system

Jun. Prof. Dr. Daniel Sauter
Institute of Molecular Virology, Ulm University Medical Center

Our body is equipped with numerous sophisticated means to combat viral infections. One important first line of defense against viruses are so-called host restriction factors. These antiviral proteins are constitutively expressed in many cell types and inhibit specific steps of the viral replication cycle. During millions of years of coevolution, however, viruses evolved effective strategies to evade or counteract these factors.

One major focus of our lab is the identification and characterization of novel restriction factors and their viral antagonists. A better understanding of the complex interplay between viruses and host will not only provide important insights into the determinants of successful viral transmission and spread, but may also uncover vulnerabilities in the viral replication cycle that can be targeted for therapeutic intervention. For example, we have previously shown that counteraction of the host restriction factor tetherin has been a prerequisite for efficient spread of HIV/AIDS in the human population. Our results also demonstrate that restriction factors often constitute significant barriers to successful transmission of animal viruses to humans. Furthermore, we identified two restriction factors that exert broad antiviral activity as they target a cellular dependency factor exploited by many major pathogens, including influenza, measles, Zika and human immunodeficiency viruses.

Guanylate-binding proteins 2 and 5 inhibit viral replication by targeting the maturation of viral envelope proteins

Maximilian Große, MSc. Student
Institute of Molecular Virology, Ulm University Medical Center

Viruses are extremely successful pathogens affecting virtually all branches of life. Since many of them lead to either cell death or oncogenesis there is high selective pressure on the host to prevent viral replication. As a result, cells evolved a variety of antiviral proteins, so-called restriction factors, which inhibit virus replication. For example, our lab has recently shown that guanylate-binding proteins (GBPs) 2 and 5 restrict HIV by interfering with maturation and virion incorporation of the viral envelope (Env) glycoprotein. In order to produce fully infectious virions, the Env precursor protein has to be cleaved by the cellular protease furin or related proprotein convertases (PCSKs). However, GBP2 and 5 suppress furin-mediated Env processing, thereby reducing infectious HIV production. By combining pull-down experiments with fluorometric reporter assays I could show that PCSK5 is also inhibited by GBP2 and 5, whereas PSCK6 and PCSK7 seem not to be affected. To elucidate the underlying mechanisms, I am currently monitoring the secretion and proteolytic activity of PCSKs in the presence or absence of GBP2 and 5. Notably, GBP2 and 5 exert broad antiviral activity as many viral pathogens exploit furin or other members of the PCSK family for the maturation of their own glycoproteins.

“To boldly go where no microbe has gone before” – fascination and responsibility of space microbiology

Dr. Ralf Möller
German Aerospace Center (DLR), Institute of Aerospace Medicine, Cologne

With international plans being formulated for solar system exploration, either using robotic probes or with human crews, microbiologists are confronted with exciting new opportunities and challenging demands. The search for signatures of life forms on another planet or moon in our solar system is one of the most prominent goals of these enterprises. Our neighbor planet Mars and Jupiter’s moon Europa are considered key targets for the search for life beyond Earth. By analogy, with terrestrial extremophilic microbial communities, e.g., those thriving in extreme environments (such as deserts) and/or those exposed to intense UV radiation, additional potential extraterrestrial habitats may be identified. Field studies with microbial communities in those extreme environments as well as microbiological studies under simulated planetary environments - in space as well as in the laboratory - will provide valuable information for preparing the “search-for-life” experiments on missions to those solar system bodies.

Another important role of microbiologists in space exploration concerns the planetary protection initiative. Here robotic orbiters, entry probes, or landers can unintentionally introduce terrestrial microorganisms to a planetary target of interest. This may destroy the opportunity to examine these bodies in their pristine condition. Depending on the target and type of mission, the planetary protection guidelines require cleaning and, in specific cases, sterilization of the spacecraft or components to avoid contamination with terrestrial organisms. The success of the cleaning and/or sterilization measures needs to be controlled by establishing a thorough inventory of the bioload prior to launch. Guidelines for bioload measurements, sterilization procedures, and effective planetary protection protocols must be established and implemented.

The presence of humans on the surface of the Moon or Mars will substantially increase the capabilities of space research and exploration; however, prior to any human exploratory mission, the critical microbial issues concerning human health and wellbeing need to be addressed. Also the need to understand evolutionary pressures exerted on microorganisms by the spaceflight environment represent additional upcoming paramount tasks for microbiologists.

In my talk, I will present data and information on previous, ongoing and future space microbiology/astrobiology activities of the DLR.

Microbial biofilms in space – Evaluating copper-based antimicrobial surfaces in the upcoming ESA space experiment BIOFILMS

Katharina Siems, PhD Student
German Aerospace Center (DLR), Institute of Aerospace Medicine, Cologne

An enduring human presence in space is required to achieve many goals of ESA´s and NASA´s space programs. During long-term missions, astronauts are exposed to various conditions such as microgravity, radiation, sleep-disruption, insufficient nutrition and also microbial contamination. Since astronauts´ immune function is compromised, all these factors increase the health risk for the crew. Therefore, improved spaceflight-suitable methods for microbiological monitoring, hygiene and decontamination are important.

Microbial biofilms are of particular concern in spaceflight, because they cannot only cause damage to equipment but also be a risk for human health. Certain metals have antimicrobial properties such as copper and its respective alloys and therefore, prevent the formation of biofilms. However, precise evaluation of suitable antimicrobial materials for space flight is essential.

Within the ESA project BIOFILMS (“Biofilm Inhibition on Flight equipment and on board the ISS using microbiologically Lethal Metal Surfaces"), effects of microgravity on biofilm formation on tailor-made nanostructured metallic surfaces are going to be examined. For that, human-associated microorganisms and antimicrobial surfaces with different chemical compositions and geometric nanostructures are selected. Experiments will be conducted aboard the ISS, where microbial growth will occur under optimal biofilm inducing conditions inside a particular designed hardware. The selected antimicrobial surfaces are supposed to be tested under different spaceflight-relevant gravitational regimes.

Here, preliminary experiments with Staphylococcus capitis for the BIOFILMS project will be presented. S. capitis forms biofilms and is related to infections such as endocarditis, urinary tract infections and catheter induced bacteremia. The reduction of growth and biofilm formation under the influence of copper-based, nanostructured surfaces was investigated.

Cell biology of malaria parasites in the mosquito

Prof. Dr. Friedrich Frischknecht
Department of Infectious Diseases, Parasitology, Heidelberg University

Malaria parasites are transmitted by Anopheles mosquitoes. The journey of the parasite starts with the uptake of Plasmodium gametocytes from the blood of an infected mammal. These ‘male’ and ‘female’ gametocytes rapidly mature to produce gametes, which fuse to form a zygote. From the zygote a motile ookinete emerges which migrates out of the blood meal and penetrates the wall of the mosquito stomach. There it builds a cyst in which it differentiates into hundreds of sporozoites. These burst out of the cyst, invade the salivary glands and can be transmitted to the skin of the mammal to start a new infection. This overview talk will illuminate some of these events with live-cell imaging and explain the function of a few known proteins that help the parasite to survive and replicate in the mosquito and hence transmit the disease.

Analysis of α-tubulin isotypic differences in Plasmodium berghei microgamete formation

Manuela Aguirre Botero, MSc. Student
Department of Infectious Diseases, Parasitology, Heidelberg University

The life cycle of Plasmodium depends on the cyclic transmission between the vertebrate host and the female mosquito vector. Transmission to the insect vector relies on the ingestion of sexual stages. Once they are in the mosquito midgut, male and female gametocytes become activated and form gametes that will later fuse and form a zygote. The male gamete is known to be one of the simplest unicellular eukaryotes and the only stage in the Plasmodium life cycle where a flagellum is assembled for motility. The rapid beating motion is essential for fertilization, making the flagellum a crucial structure for the successful proliferation of this parasite.

The core structure of the flagellum is the axoneme, a microtubular formation that is formed from α-tubulin and β-tubulin heterodimers. Microtubules are not only essential for the formation of the flagellum but also for the cell shape and cell division. Interestingly, Plasmodium expresses two α-tubulin isoforms. α-tubulin I is required for the motility and shape of sporozoites, the stage that is transmitted to the vertebrate host. On the other hand, α-tubulin II has been shown to be highly expressed in male gametes. Substitution of α-tubulin II with the open reading frame of α-tubulin I resulted in a strong impairment of gamete assembly. Transmission electron microscopy (TEM) and electron tomography (ET) revealed that this mutant has abnormal axonemal structures. Consequently, mutants containing α-tubulin II complemented with α-tubulin I fragments are used to further understand the isotypic differences of α-tubulin and their role in the axonemal formation and the subsequent gamete function.

Development of an optimized DNA and RNA-based murine norovirus reverse genetics system

Prof. Dr. Stefan Taube
Institute for Virology and Cell Biology, University of Lübeck

Marit Lingemann, PhD Student
Institute for Virology and Cell Biology, University of Lübeck

Noroviruses are the leading course of non-bacterial acute gastroenteritis leading to millions of infections each year worldwide. Due to limitations to efficiently cultivate human norovirus (HuNoV), murine norovirus (MNV) has become a relevant and efficient model to study norovirus infection in cell culture and in its natural host. A reverse genetics systems allows for introducing targeted mutations into the MNV genome and study the impact on infection. The recently proteinaceous entry receptor (murine CD300lf) is sufficient to mediate susceptibility to MNV infection in various eukaryotic cell lines. We investigated, whether this allowed for improved production of recombinant MNV by reverse genetics in various cell lines. For this we use the Huh7 cell-line and derivatives (Huh7.5 and Huh7lunet) transduced with a lentivirus vector providing the entry receptor. Virus titers were determined by end-point dilution and plaque-assay. To facilitate high quality virus titrations in different cell lines, we adapted a microcrystalline cellulose (Avicel)-based plaque- assay originally developed for influenza virus titrations. Overall we present a simple and efficient platform to generate recombinant MNV from cDNA and RNA and perform titrations using microcrystalline cellulose based plaque-assays.

Vaccination, Vaccines, and Vaccination Recommendations

Prof. Dr. Thomas Mertens
Institute for Virology, Ulm University Medical Center/
Robert Koch Institute (STIKO), Berlin

The problem of well-known and emerging infectious diseases can be only definitely resolved if an efficient vaccine is made available. This remains true in the area of antimicrobial and antiviral therapy which reduce the mortality rate and mortality, but do not provide major effects on the epidemiology of pathogens. So, the development of vaccination and vaccines using the natural defense mechanisms and reducing incidence and prevalence of infections is (one of) the most important achievement(s) of medical research for the health of individuals and human populations. The role of the different effector functions involved in the protection after vaccination is dependent on agents and vaccines. The story of the success of vaccination is long and impressive:

- Worldwide eradication of **Human variola virus** (1977). In 1966 ca. 10-15 Mio. human pox patients in 31 endemic countries.
- Elimination of **Poliomyelitis** in nearly all countries. In 1952 9500 patients suffered from Poliomyelitis in Germany and 745 died.
- Major Reduction of **Hepatitis B** in children.
- No more cases of **Diphtheria and Tetanus** in Germany.
- Reduction of **Measles Mumps and Rubella**. From 2000 to 2010 the global number of Measles fatalities dropped from 535.300 to 139.300 (Vaccine coverage about 85%).

The strategic aims of vaccination are protection of the vaccinee, herd-immunity or even eradication, depending on the pathogen. Eradication is only possible, if the pathogen does not possess extra- human reservoirs.
Public interest can be defined for all mentioned vaccination aims. After development of a new vaccine, the efficacy and safety has to be thoroughly proven, vaccination recommendation must be developed based on the best available evidence and considering the disease burden, the implementation and the costs.
In Germany by law the standing committee on vaccination recommendations, an independent scientific committee (STIKO), must develop these recommendations and monitor the effects after implementation.

Bacteria interact with surrounding cells... ... at war with each other

Prof. Dr. Daniel Unterweger
Max Planck Institute for Evolutionary Biology, Plön
Institute of Experimental Medicine, Kiel University, Kiel

Bacteria are omnipresent in medicine. Either as causative agent of potentially life-threatening infections or as inhabitants of our body that contribute to our well-being on a daily basis. Given their abundance, bacteria pose two key challenges to human health. Firstly, rising levels of antimicrobial resistance decrease our treatment options for bacterial infections. Secondly, we realize the important role of bacteria for our body without understanding how these bacteria live and how we could manipulate them to our benefit.

We study bacteria interacting with other bacteria and the host. One project focuses on a macromolecular machinery with which bacteria kill each other, the type VI secretion system. The goal of this project is to understand the molecular mechanisms by which bacteria interact with each other in densely packed communities.

Bacteria interact with surrounding cells... ... as evolutionary driver of the host

Dr. Marie Vallier
Max Planck Institute for Evolutionary Biology, Plön

B4galnt2 is a blood group-related glycosyltransferase whose two murine alleles (driving gastrointestinal and vascular expression) are maintained by balancing selection in house mice and their relatives. The vascular allele induces a defect in coagulation and subsequent bleeding phenotype, suggesting that this fitness cost may be offset by other unknown benefits. Interestingly, despite its overall long-term maintenance, the vascular allele is absent in wild mouse populations from Germany and Northeast France, but recently increased in Southwest France as evidenced by a partial selective sweep. This suggests that geographic-dependent selective forces may be operating.

Given other examples of blood group-related glycosyltransferase variation in humans, we hypothesize that resistance to pathogen(s) may mediate selection operating on B4galnt2 over space and time. Indeed, experiments in lab mice show that the presence/absence of B4galnt2 expression in the gastrointestinal tract influences the response to Salmonella infection. By applying metagenomic approaches in a wild mouse population displaying evidence of recent selection (i.e. a partial selective sweep), we found that B4galnt2 genotype correlates with differences in intestinal inflammation and the presence of candidate pathogens that could drive selection at B4galnt2. We thus suggest the limited dispersal of pathogens to be a potentially potent source of variation in selection.

Structural basis for the switch between RNA replication and virion morphogenesis in pestiviruses

Prof. Dr. Norbert Tautz
Institute of Virology and Cell Biology, University of Lübeck

Pestiviruses like bovine viral diarrhea virus (BVDV) and classical swine fever virus (CSFV) cause economically highly relevant diseases in livestock industries. On the molecular level pestiviruses show a high degree of similarity to the human pathogen hepatitis C virus (HCV).

The positive-strand RNA genome of pestiviruses is translated into one large polyprotein which is processed by cellular and virus encoded proteases into twelve functional proteins. Four structural proteins are present in the infectious virus particles. However, virion formation requires besides the structural proteins also most of the nonstructural proteins (NS). Accordingly, these NS proteins have to function in two basically different processes, namely genome replication and virion morphogenesis. Our research aims at the question how this multifunctionality is realized on the molecular level.

An important feature of pestiviruses is their capacity to establish persistent infections in their animal host. A prerequisite for persistence is a sophisticated mechanism to restrict viral RNA replication efficacy in the infected cell. Viral persistence often ends with a sudden death after a long persistent period. Lethal disease is caused by the emergence of deregulated virus mutants containing cell-derived mRNA sequences in their genomes due to RNA recombination. We are working on the characterization of the “molecular brake” as well as the phenomenon “RNA recombination” and its consequences.

Determination of the membrane topology of non-structural protein 2 (NS2) of pestiviurses

Thomas Walther, PhD Student
Institute of Virology and Cell Biology, University of Lübeck

BVDV is an important pathogen of livestock that belongs to the genus Pestivirus of the family Flaviviridae. The BVDV life cycle is characterized by a complex interplay between viral and host factors, in association with cytoplasmic membranes. The cleavage of nonstructural protein 2-3 (NS2-3), catalyzed by an autoprotease in NS2, is a crucial regulatory step for these viruses. While the released NS3 is an essential component of the viral RNA replicase complex uncleaved NS2-3 is required for virion morphogenesis. Regulation of NS2-3 cleavage is used by pestiviruses to restrict their RNA replication efficacy.

For a better understanding of these processes, a detailed knowledge of the structural features of pestiviral NS2 and NS2-3 is mandatory. To elucidate the membrane topology of BVDV NS2, we established the “substituted cysteine accessibility method” (SCAM) assay to map cytoplasmic regions of its N-terminal hydrophobic domain. Furthermore, we applied an in vivo glycosylation assay to determine the respective luminal regions. The characterizations indicate that BVDV NS2 has three transmembrane segments (TMS). The experimental topology determination confirmed the luminal localization of the N-terminal part as well as the cytoplasmic localization of the C-terminal protease domain. While these overall features resemble HCV NS2, some interesting differences were identified which are subject to future research.

Notes

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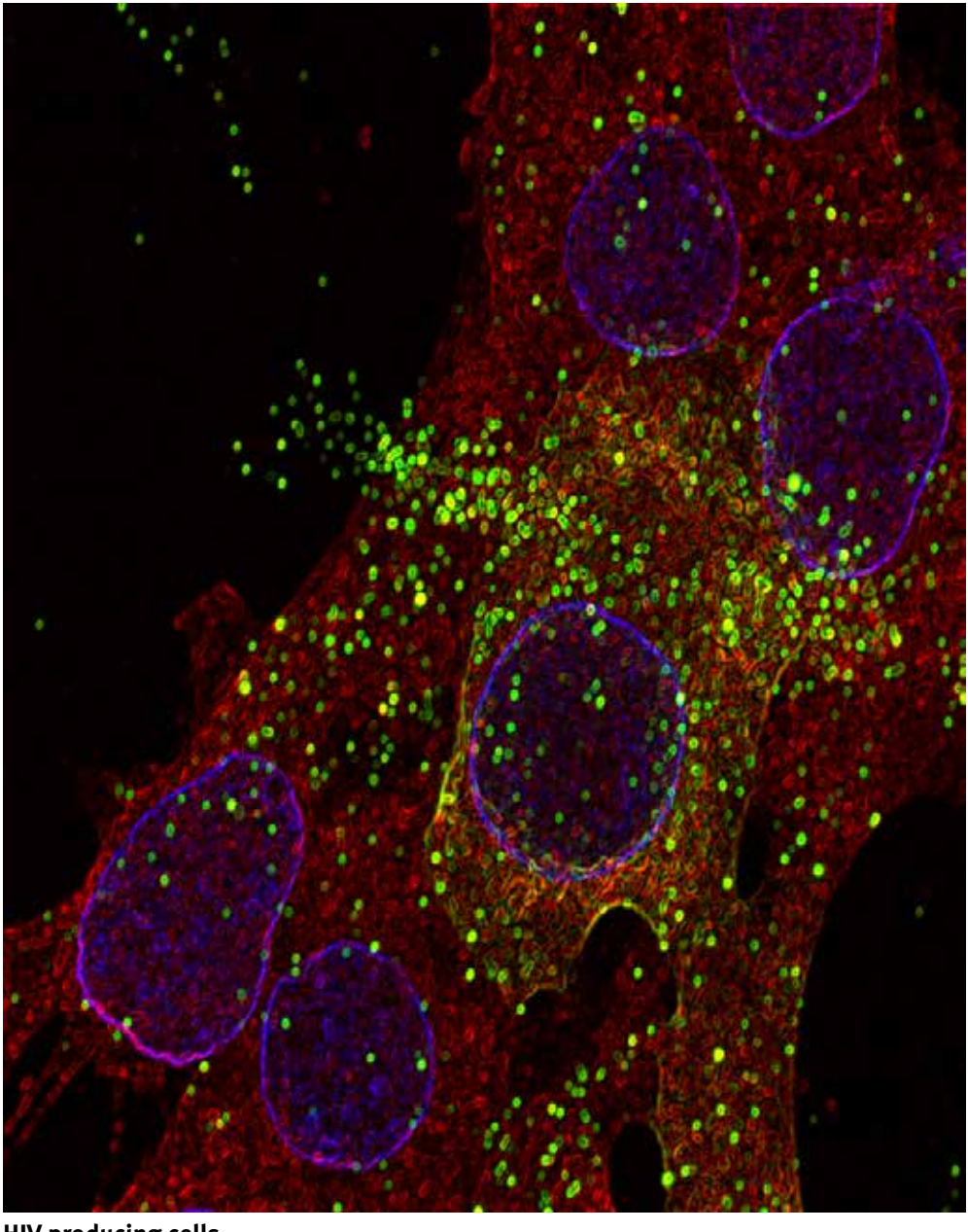

HIV producing cells
Jun. Prof. Dr. Daniel Sauter, Institute of Molecular Virology, Ulm University Medical Center

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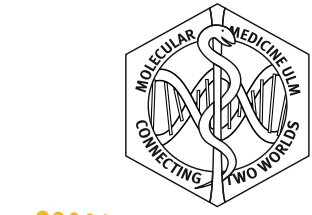

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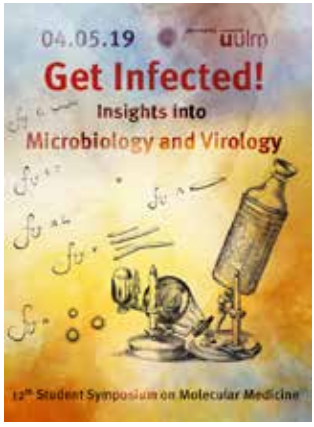

Title image: Robert Hooke Microscope and drawings of bacteria (originally by van Leeuwenhoek) and mimi virus, created by Vivien Hoch.