Institute of Molecular Virology and Cell Biology of the Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health

1. Task description

The Institute of Molecular Virology and Cell Biology (IMVZ) (formerly the Institute of Molecular Biology) is one of eleven specialist institutes of the Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health (FLI), an independent higher federal authority and governmental research institute within the portfolio of the Federal Ministry of Food and Agriculture (BMEL). Within the scope of its legal tasks, which are mainly laid down in Article 27 of the Animal Health Act, the Institute of Molecular Virology and Cell Biology investigates the mechanisms of interaction of infectious pathogens with host cells and organisms, with special emphasis on virus infections of farm animals. Classical virological, molecular biological, biochemical, mass spectrometric and imaging methods are used. Research conducted at the institute serves to elucidate the basic mechanisms of viral infection processes as a basis for the development of strategies to prevent infection-related damage to animal health and the transmission of pathogens from animals to humans (zoonoses). The institute advises the BMEL in these areas.

Since the University of Greifswald, Faculty of Mathematics and Natural Sciences, strives to strenghten education in the field of virology, a joint appointment is planned.

2. Fields of activity and main areas of work

Knowledge of the molecular processes involved in the interaction between infectious agent and host is a fundamental prerequisite for understanding the complex interactions leading to infection and the development of disease symptoms. It is also the basis for the development of new preventive and therapeutic methods. This includes the molecular biological investigation of pathogens with regard to their genetic makeup and the elucidation of the function of viral gene products. In the past, this has led, for example, to the introduction of marker or DIVA vaccines, which have revolutionised animal disease control. In contrast, the manifold virus-host cell interactions are insufficiently known due to the complexity of the hosts. However, knowledge of these interactions at the molecular level is an essential basis for novel methods of prevention and control of animal diseases and zoonoses that go beyond the vaccines and therapeutics available to date. By combining molecular biological techniques such as reverse genetics, mass spectrometric analyses and modern microscopic methods such as confocal, live cell and light sheet fluorescence microscopy, viral gene products are functionally characterized, infection-induced changes in the protein pattern of the host are detected, and the course of infections in cell cultures and complex host tissues is investigated. The combination of the key technologies established at the IMVZ enables comprehensive molecular analyses of infection biological and pathogenetic processes, with expertise in bioinformatics gaining importance in terms of increasing digitization, scope and complexity of data obtained.

The spectrum of pathogens includes in particular notifiable animal disease pathogens and zoonotic viruses. In addition to pathogens of the highest risk group 4 such as ebola- and henipaviruses, there is a special focus on neurotropic viruses and infectious agents of the respiratory tract. In addition to basic questions, tasks within the framework of animal disease diagnostics (National Reference Laboratories for Rabies, Aujeszky's Disease and Infectious Laryngotracheitis) are also performed. In addition to classical cell culture systems, relevant primary cell models and modern methods for the detection of virus-host interactions in infected animals are being developed for the various pathogens.

The Institute of Molecular Virology and Cell Biology addresses the following topics:

2.1. Rabies

While terrestrial rabies is considered extinct in Western Europe, it still is the most important zoonosis in other parts of the world. Each year, more than 70,000 people worldwide die from the infection, which in most cases is transmitted by injuries after contact with dogs. In addition, new rabies viruses are increasingly detected in reservoir hosts, in particular bats. Knowledge of the spectrum of naturally occurring viruses and understanding of their interaction with different hosts will provide the basis for the improvement of vaccines and the protection of humans from infection. Therefore, rabies research of the FLI has been consolidated at the IMVZ. The National Reference Laboratory for Rabies, the OIE Reference Laboratory, and the WHO Collaborating Centre for Rabies Surveillance and Research are part of the institute, as is the Working Group on Molecular Biology of Rabies Viruses, so that practical aspects of rabies control, understanding of the pathogenesis of rabies in the infected host and the molecular basis of this interaction can be investigated in close collaboration.

2.2. Aujeszky’s Disease (AD, Pseudorabies)

The notifiable AD is a herpesvirus infection of swine, which also affects other mammalian species with the exception of higher primates, including humans. AD in domestic pigs in Germany was eradicated by using marker vaccines for the first time (DIVA principle), but is still present in other European and non-European countries. Despite considerable progress in eradication, infections in wild boar populations are increasing drastically. At the institute, work on AD in the frame of molecular biological research and of the National Reference Laboratory has been consolidated. Fundamental research deals with the interaction of viral and cellular components in virus morphogenesis in order to elucidate and, if necessary, specifically inactivate cellular metabolic and transport pathways necessary for virus replication. In cooperation with other research groups, the structure of selected viral proteins is also made visible and analyzed. In addition, pseudorabies viruses from wild boar are characterized molecular biologically and with regard to their pathogenesis.

2.3. Influenza

Influenza plays an important role both in the field of animal diseases (avian influenza, „bird flu“, swine influenza) and in the field of public health („swine flu“). In the sense of a „One Health strategy“, research into pathogenic properties based on molecular virus-host interactions has created important foundations for novel vaccines and knowledge-based risk analyses. At the IMVZ, disease- and host-determining factors are characterized on the basis of recombinant porcine and avian influenza viruses (reverse

genetics) and mechanisms of host-host interaction are investigated. The focus is on the transformation of low pathogenic avian influenza viruses into highly pathogenic fowl plague pathogens and on the species transition from *Anatidae* to *Galliformes* and from animal hosts such as birds or swine to humans.

2.4. African Swine Fever

African Swine Fever (ASF), originally confined to Africa, has spread in recent years mainly in Eastern Europe and has now crossed the borders of the EU. To date, neither vaccines nor therapeutics against this animal disease are available. The IMVZ has initiated basic research to understand ASF infection and new approaches for the development of vaccines. As a phylogenetically isolated, complex DNA virus, the ASF pathogen represents a research object that is not yet adequately understood. The aim of the investigations is to gain a better understanding of the biology of ASF and the importance of viral proteins for the replication cycle of the virus, for the immune response of the host, and for its suppression (immune evasion). To this end, the extensive expertise of the institute in the investigation of similarly complex DNA-containing herpesviruses using modern virus- and host genome-manipulating techniques such as CRISPR/Cas is applied.

2.5. Newcastle Disease

Newcastle Disease (ND) or atypical fowl plague is a notifiable infectious virus disease that can lead to high economic losses in farmed poultry. In Germany, prophylactic vaccination of domestic *Galliformes* is mandatory. One of the first reverse genetic systems for the pathogen NDV was established at the IMVZ. On this basis, research is conducted to gain a basic understanding of the functions of viral gene products and their interaction with the host cell. NDV vectors are also used to develop new approaches for vaccination against other poultry diseases, such as avian influenza.

2.6. Infectious Laryngotracheitis

In Germany, Infectious Laryngotracheitis (ILT) is a reportable herpesvirus infection of chickens, which as rule causes only moderate economic damage, but has not yet been eradicated despite the availability of effective vaccines. The IMVZ houses the National Reference Laboratory for ILT.

2.7. Filoviruses

Filoviruses (ebola-, Marburg- and cuevaviruses) are zoonotic viruses, which can cause haemorrhagic fever with high mortality in humans. As pathogens of the highest risk group 4, infectious filoviruses can only be investigated in high-containment laboratories. Ebolavirus infections are notifiable in Germany. While most filovirus outbreaks occur in Africa, cuevaviruses, which are closely related to ebolaviruses and whose pathogenic potential is currently unknown, have also been found in Europe (Spain and Hungary). The IMVZ investigates virus-host interactions and pathogenicity mechanisms, in order to find new targets for broad-spectrum therapeutics and to collect basic knowledge for evidence-based risk assessments of new filoviruses. A further focus is the identification and characterization of novel filoviruses, as well as the development and application of model systems that allow research on filoviruses and testing of therapeutics outside high containment laboratories.

2.8. Henipaviruses

Hendra and Nipah viruses are zoonotic agents of risk group 4 that cause severe, often lethal infections of the respiratory tract and central nervous system in farm animals (pigs and horses) and humans. The IMVZ conducts research on molecular virus-host interactions and establishes new reverse genetic systems for the investigation of henipaviruses in the S4 high containment laboratory of the FLI. Research focuses on basic analyses of pathogen replication and the involvement of cellular host factors and their relevance in animal models using new 3D imaging approaches.

2.9. Confocal, live cell Imaging and light sheet fluorescence microscopy

The analysis of viral replication processes by high-resolution fluorescence microscopy represents a new dimension in the visualization of the interaction between pathogen and host. At the IMVZ, intra- and intercellular maturation and transport processes are investigated by live cell imaging in combination with electron microscopic ultrastructural imaging. Modern laser scanning microscopes with high speed live imaging equipment are available for protection levels 2, 3, and 4. The results obtained provide fundamental insights into pathogen-host cell interactions, virus spread and possible intervention options. The use of primary cells such as isolated neurons or Airway Liquid Interphase (ALI) cultures from primary lung epithelial cells allows the investigation of the infection dynamics of neutrophic and respiratory viruses in relevant semi-*in vivo* infection models. Virus replication and pathogenetic processes in tissues and organs are recorded by light microscopy using 3D imaging of optically clarified samples and are evaluated quantitatively. This allows the analysis of intracellular processes directly in the infected tissue and, in the future, will allow the imaging of infection processes in complete small animal organs and larger tissue areas using novel light sheet fluorescence microscopy.

2.10. Mass spectrometry

The FLI houses the mass spectrometry platform of the FLI. It is equipped with a powerful MALDI mass spectrometer with MALDI imaging equipment, which allows spatially resolved mass spectrometric analysis of tissue sections. The investigation of the proteome of a cell or tissue and the changes after infection enables detailed mapping of the cellular processes manipulated by pathogens and thus the identification of target structures for preventive and therapeutic interventions. Proteome analyses also play an outstanding role in the search for cellular interaction partners of viral components. In bacteriological diagnostics, MALDI-TOF mass spectrometry is used increasingly for rapid pathogen identification and species identification.

2.11. Medium-term aims

The aim is to strengthen research in the field of cell biology as a basis for a more comprehensive understanding of pathogen-host interactions. The results obtained are relevant for more targeted intervention measures with novel mechanisms of action and an improved risk analysis. In addition to broadening research on highly pathogenic agents of risk groups 3 and 4, it is intended to increasingly conduct research on molecular and cell biological aspects of infection and pathomechanisms in relevant *in vivo* and primary cell infection models. This will take into account the importance of

more complex interactions such as cell tropism, intra- and extracellular host factors including immune reactions and immunopathogenesis for pathogen biology.

Methodologically, a combination of proteomics with other „omics“ technologies (genomics, transcriptomics) for system biological analysis of virus-host interactions is planned. With the establishment of novel sample processing and imaging methods such as uDISCO and light sheet fluorescence microscopy, current efforts for the *ex vivo* analysis of complex tissues with cellular and subcellular resolution are to be advanced and bioinformatic methods for the visualization and quantitative analysis of 3D image data sets are to be strengthened.

3. Collaboration

The Institute of Molecular Virology and Cell Biology maintains close cooperation with the other specialist institutes of the FLI. This is also reflected in joint projects and is of great importance for addressing complex questions in case of infection events, in which the expertise of the individual specialist institutes is used. In part, this is specifically supported by FLI-internal research alliances. Alliances on virus-host interactions and pathogenesis of zoonotic viruses of risk group 4 and on pathogenesis, diagnostics, and surveillance of lyssaviruses are coordinated by the IMVZ. Collaboration within the FLI provides the opportunity to use key technologies such as electron microscopy and next-generation sequencing.

In addition, various cooperations exist at national and international level with universities and research institutions. A number of these cooperations result from consortium projects funded by the DFG, BMBF, EU or private companies. The acquisition of funding from the German Research Foundation (DFG) is regarded as essential by the institute for successfully addressing its research questions.

Cooperation exists with the following institutes:

national:

- University of Greifswald
- Free University of Berlin
- IDT Biologika Dessau/Riems
- Paul-Ehrlich-Institut, Langen
- Philipps-Universität Marburg
- Heinrich Pette Institute Hamburg
- University of Veterinary Medicine Hannover
- Leipzig University
- Justus Liebig University Giessen

international:

- Animal and Plant Health Agency, Weybridge, UK
- CRESA, Barcelona, Spain
- Friedrich Miescher Laboratory of the Max Planck Society, Tübingen
- INRA, Jouy-en-Josas, France
- Institut Pasteur, Paris, France
- International Livestock Research Institute, Nairobi, Kenya

- Leeds University, UK
- MSD, Boxmeer, The Netherlands
- Tel Aviv University, Israel
- The Pirbright Institute, UK
- Ghent University, Belgium
- University of Lisbon, Portugal
- University of Oxford, Oxford Particle Imaging Centre, UK
- Damanhur University, Egypt
- Njala University, Sierra Leone
- Sierra Leone Agricultural Research Institute (SLARI), Sierra Leone
- Institut Pasteur Conakry in Guinea
- Gifu University, Japan

4. Education

Scientists of the institute primarily teach at the University of Greifswald. The head of the institute will be appointed jointly with the University of Greifswald. The tasks include education in virology at the Faculty of Mathematics and Natural Sciences of the University of Greifswald with a teaching load of two semester hours per week.

5. Publications of the Institute of Molecular Virology and Cell Biology:

Selected publications of the Institute of Molecular Virology and Cell Biology of the past 3 years:

- Keßler C, Forth JH, Keil GM, Mettenleiter TC, Blome S, Karger A. (2018) The intracellular proteome of African swine fever virus. Sci Rep.

- Hübner A, Petersen B, Keil GM, Niemann H, Mettenleiter TC, Fuchs W. (2018) Efficient inhibition of African swine fever virus replication by CRISPR/Cas9 targeting of the viral p30 gene (CP204L). Sci Rep.

- Zaeck L, Potratz M, Freuling C M , Muller T , Finke S. (2019) High-Resolution 3D Imaging of Rabies Virus Infection in Solvent-Cleared Brain Tissue. J Vis Exp.

- Hassel R, Vos A, Clausen P, Moore S, van der Westhuizen J, Khaiseb S, Kabajani J, Pfaff F, Hoper D, Hundt B, Jago M, Bruwer F, Lindeque P, Finke S, Freuling CM, Muller T. (2018). Experimental screening studies on rabies virus transmission and oral rabies vaccination of the Greater Kudu (Tragelaphus strepsiceros). Sci Rep.

- Eggerbauer E, Pfaff F, Finke S, Hoper D, Beer M, Mettenleiter TC, Nolden T, Teifke JP, Muller T, Freuling CM. (2017). Comparative analysis of European bat lyssavirus 1 pathogenicity in the mouse model. PLoS Negl Trop Dis.

- Martin S, Chiramel AI, Schmidt ML, Chen YC, Whitt N, Watt A, Dunham EC, Shifflett K, Traeger S, Leske A, Buehler E, Martellaro C, Brandt J, Wendt L, Müller A, Peitsch S, Best SM, Stech J, Finke S, Römer-Oberdörfer A, Groseth A, Feldmann H, Hoenen T. (2018) A genome-wide siRNA screen identifies a druggable host pathway essential for the Ebola virus life cycle. Genome Med.

- Kämper L, Zierke L, Schmidt ML, Müller A, Wendt L, Brandt J, Hartmann E, Braun S, Holzerland J, Groseth A, Hoenen T. (2019) Assessment of the function and intergenus-compatibility of Ebola and Lloviu virus proteins. J Gen Virol.

- Vallbracht M, Brun D, Tassinari M, Vaney M-C, Pehau-Arnaudet G, Guardado-Calvo, P, Haouz A, Klupp BG, Mettenleiter TC, Rey FA, Backovic M. (2018) Structure-Function Dissection of Pseudorabies Virus Glycoprotein B Fusion Loops. J Virol. 92 (spotlight)

- Klupp BG, Hellberg T, Granzow H, Franzke K, Dominguez Gonzalez B, Goodchild RE, Mettenleiter TC. (2017) Integrity of the Linker of Nucleoskeleton and Cytoskeleton Is Required for Efficient Herpesvirus Nuclear Egress. J Virol.

- Dittrich A, Scheibner D, Salaheldin AH, Veits J, Gischke M, Mettenleiter TC, Abdelwhab EM. (2018) Impact of Mutations in the Hemagglutinin of H10N7 Viruses Isolated from Seals on Virus Replication in Avian and Human Cells. Viruses

- Karsunke J, Heiden S, Murr M, Karger A, Franzke K, Mettenleiter TC, Römer-Oberdörfer A. (2019) W protein expression by Newcastle disease virus. Virus Res.

- Pauker VI, Bertzbach LD, Hohmann A, Kheimar A, Teifke JP, Mettenleiter TC, Karger A, Kaufer BB (2019). Imaging mass spectrometry and proteome analysis of Marek's disease virus-induced tumors. mSphere 4