One hundred years of animal virology

Rudolf Rott¹ and Stuart Siddell²

¹ Institute for Virology, Justus Liebig University Giessen, 35392 Giessen, Germany
² Institute of Virology and Immunology, Julius Maximilian University Würzburg, 97078 Würzburg, Germany

Introduction

In June 1998, the Ernst Moritz Arndt University, Greifswald, Germany, and the German Federal Research Centre for Virus Diseases of Animals organized a centenary meeting to celebrate ‘One Hundred Years of Animal Virology’ in Germany. The meeting was held at the birthplace of German virology; the University of Greifswald and the Friedrich Loeffler Institutes on the Island of Riems in Mecklenburg-Vorpommern. It was here that the then Professor of Hygiene, Friedrich Loeffler, carried out much of his pioneering work on the agent that causes foot-and-mouth disease (FMD).

An historical perspective

‘Members of the undersigned Commission obediently inform Your Excellency of the results of their investigations thus far obtained on foot and mouth disease.’ This is the opening sentence of the extensive report given in 1898 to the Prussian Ministry of Culture by a Research Commission on FMD headed by Friedrich Loeffler, together with his colleague Paul Frosch from Robert Koch’s Institute of Infectious Diseases in Berlin (Loeffler & Frosch, 1898). The Commission had been set up the previous year by the Prussian Ministry of Culture, following a request from the ‘Partei der Landwirte’ of the ‘Reichstag’. The Prussian State had a special interest in the battle against FMD, since at that time it was causing severe economic damage to the country’s agriculture. The characteristic symptoms of FMD, which affects cloven-hoofed animals with high morbidity but low mortality, are high fever, reduction in milk production and vesicular lesions which develop 2–8 days after infection. These lesions are principally found in and around the mouth, in the interdigital skin, on the coronary bands of the feet and on the teats.

The avowed task of the Commission was to develop a vaccine which would prevent further outbreaks of the disease. In the aforementioned report, Loeffler and Frosch described their discovery that the causative agent of FMD was neither a known bacterium nor a toxin but an ‘ultravisible, ultrafilterable substance’ which previously had gone unnoticed due to its minute size. In the extensive and very precise experiments undertaken, they were able to transmit the disease from calves suffering from FMD to other susceptible cloven-hoofed animals by transplanting germ-free filtered vesicle lymph.

At that time, the porous-clay filters developed by Edwin Klebs from Königsberg were mainly used to produce germ-free and thereby hygienic and safe drinking water. In 1888 however, Emile Roux, a disciple of Pasteur, and his Swiss colleague Alexandre Yersin had discovered that with certain infectious diseases, e.g. diphtheria or tetanus, bacterial sterile filtrates could still produce typical clinical symptoms in laboratory animals. With this observation, they had proven that there are bacteria which produce and excrete a disease-determining toxin. This discovery, coupled with the later finding of the so-called anti-toxin (which eventually lead to the development of Emil von Behring’s serum therapy), would have practical consequences previously undreamed of.

In 1892 the Russian biologist Dimitri I. Ivanovski had described that the causative agent of tobacco mosaic disease was a filterable agent. Probably prejudiced by the findings on the bacterial toxin of diphtheria, Ivanovski was led to believe that the causative agent of tobacco mosaic disease is a toxin-producing bacterium. Ivanovski’s findings were emphatically confirmed in 1898 by Martinus W. Beijerinck, a Dutch soil microbiologist, who went on to show, however, that the agent could not be cultivated in non-living media, as would be expected for a bacterium. Moreover, the agent could be multiplied in infected tissue and therefore could not be a toxin. To explain filterability, Beijerinck concluded that the agent was a ‘contagium vivum fluidum’ which means a not precisely defined contagious, reproducible liquid entity. At the same time, Loeffler and Frosch were able to rule out a toxin as the cause of FMD by serial transmission of filtered vesicle from diseased animals. According to the meticulous calculations which they made, if the cause of FMD was toxin-based, then after several animal-to-animal transmissions the original material would be so diluted that only ‘1:2⁵ trillion’ of the starting substance would remain. ‘A toxic effect of that nature would be unbelievable’ they concluded. Since the disease ran its characteristic course with every round of transmission, the causative agent, therefore, must be some sort of minute agent capable of reproducing itself.

Furthermore, Loeffler and Frosch already knew from their initial studies that the infection of animals resulted in a solid
immunity. Analogous to Behring's anti-toxin therapy, they found, in the blood of immune animals, substances which neutralized infectivity and the pathogenic effect of the causative agent, i.e. they found antibodies which protected against infection. Loeffler and Frosch reported in the "Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten" (Loeffler & Frosch, 1897) 'that cows and sheep can be artificially immunized. Immunization can be achieved by injecting filtered vesicle fluid which has first been sufficiently pre-heated to destroy infectivity, or by injecting a vesicle lymph/immune blood mixture, or passively by the serum of immune calves. The overwhelming majority of animals will be immune after just one single injection. The protective injection obviously did not make the animals ill. It is therefore scientifically proven that one can effectively fight against foot-and-mouth disease by protective immunization.' Loeffler and Frosch were, therefore, the first to use an inactivated vaccine to fight a virus infection. With these results, the Commission could effectively regard its task as being complete. This could also be deduced from their comment: 'Because of the ultravisibility and the inability to grow the causative agent in artificial cultures, the research work (will), for the time being, be brought to an end; the budget should not be overspent.'

In view of their contribution, Loeffler and Frosch can justifiably be regarded as the founders of animal virology. Most certainly they must have been aware that they had opened the door to a whole new area of research. At the end of their report, they assessed their discovery as follows: 'If further experiments carried out by the Commission confirm that the filtrate effects are, as it indeed appears to be the case, in fact caused by minute living things, then one could very well assume that the causative agents of numerous other infectious diseases of man and animals such as pox, cowpox, scarlet fever, measles, typhus, rinderpest, etc., which up until now have all been searched for to no avail, may also belong to this group of minute organisms. The germ-free filtrates of the infectious substances would be the appropriate source to gain new important information as to the nature of the disease in question.' Three years later the 'ultravisible and filterable causative agents' (nowadays known as viruses) of fowl plague and yellow fever had been discovered and similar discoveries in many more human and animal diseases were to follow.

A contemporary perspective

Perhaps not surprisingly, the first virus to take the stage at the 1998 meeting was FMDV. Fred Brown (Plum Island, USA) described recent efforts to develop a synthetic peptide vaccine which protects against multiple serotypes of the agent and Andy King (Pirbright, UK) described the VP1 antigenic site, which forms the core of such vaccines, and its interaction with RGD-binding integrins, the major class of FMDV cellular receptors. Interestingly, under appropriate conditions, infection by most FMDV strains can also be shown to depend upon heparin sulphate receptors. The crystal structure of heparin bound to a high affinity strain of subtype O1 reveals three sugar residues bound tightly and specifically in a groove on the capsid surface. This groove is made up of all three surface proteins and is structurally analogous to the receptor-attachment 'pit' of the related cardioviruses. The role of heparin sulphate proteoglycans as FMDV receptors in vivo remains unclear. Eckard Wimmer ( Stony Brook, USA) outlined the hallmarks of poliovirus replication; namely the initiation of protein synthesis by an IRES element, the formation and processing of the polyprotein and the initiation of RNA synthesis by protein priming. He then turned to recent evidence that the neuropathogenesis of poliovirus is determined, at least partially, by cell type-specific IRES function and dependence on the developmentally expressed human poliovirus receptor, CD155. In allusion to the WHO eradication programme, Professor Wimmer's bitter-sweet conclusion is that 'Poliovirus is a virus with a brilliant past and a fascinating present, but with no future'.

A second theme of the meeting was the interaction between viruses and the immune system. Volker ter Meulen (Würzburg, Germany) described the profound immune suppression resulting from measles virus (MV) infection. In vivo studies show that freshly isolated, uninfected peripheral blood lymphocytes are refractory to mitogen stimulation after surface contact with UV-inactivated cells expressing the MV glycoproteins, F and H. Interestingly, dendritic cells, which undergo a rapid functional maturation after MV infection, reveal a strongly impaired allostimulatory activity in a mixed leukocyte reaction and actively prevent mitogen-dependent proliferation of allogeneic and autologous lymphocytes. The immunomodulatory capabilities of viruses were further highlighted by Geoffrey Smith (Oxford, UK) who described a series of poxvirus gene products with immunomodulatory properties. These included the vaccinia virus B15R, vCKBP and B18R proteins which are secreted from the infected cell and bind cytokines, chemokines or interferons. The study of these virus immunomodulatory proteins is enhancing our knowledge of virus pathogenesis, yielding fundamental information about the function of host factors and providing new molecules that have potential application for the treatment of immunological disorders or infectious disease. In a similar vein, Bernhard Fleckenstein (Erlangen, Germany) noted that human herpesvirus type 8 (HHV-8) encodes a number of proteins that are predicted to be (1) a functional but deregulated D-type cyclin, (2) cytokines, a cytokine effector and an interleukin-8 receptor type B, (3) anti-apoptotic proteins, (4) anti-complement proteins and (5) enzymes of nucleotide metabolism. It appears that the effectors of HHV-8 cell transformation may be encoded, at least in part, by the sequestered, cell-derived genes that are non-essential for virus replication. Harriet Robinson (Atlanta, USA) brought the meeting up to date on the use of DNA vaccines and discussed the merits, and pitfalls, of
different delivery strategies. Evidently, although the parameters involved are complex, the immune response can now be biased towards type-1 or type-2 T cell help by the method of DNA delivery, the form of the expressed antigen and co-delivered immunostimulatory molecules. DNA vaccines for HIV, Plasmodium spp., influenza virus, herpesviruses and hepatitis B virus are all in an advanced stage of development. Reinhard Kurth (Langen, Germany) reminded us that, despite years of intense study, the precise reasons for the demise of the immune system characteristic of HIV infection of humans and SIVmac infection of monkeys remains unknown. The viruses SIVagm and SIVsm, both of which have been shown to cause immunodeficiency in heterologous species, do not induce disease in their respective natural hosts. This indicates that the natural hosts are not susceptible to the immunosuppressive effect of the virus and provides hope that new opportunities for therapy and prophylaxis exist, if only we can elucidate the mechanisms of resistance.

The third major area covered at the meeting was the threat posed by emerging and re-emerging virus infections in both the developed and developing world. Brian Mahy (Atlanta, USA) noted that during the last 15 years, more than 40 viruses which are capable of infecting humans (sometimes inapparently, e.g. hepatitis G virus; sometimes with devastating consequences, e.g. HIV) have been newly identified. At the same time, there has been a resurgence of previously known virus diseases such as dengue and Ebola haemorrhagic fever. By and large, the viruses of concern are RNA viruses with high mutation rates and the opportunity for genetic reassortment or recombination. This fact, combined with changes in human population and social behaviour, new methods of livestock husbandry, the increased number of immunocompromised individuals and the resurgence of arthropod vectors, can lead us to expect an unprecedented explosion of emergence and recognition of new virus infections. This conclusion was emphatically underlined by both Robert Webster (Memphis, USA), who described the recent emergence of the avian H5N1 strain of influenza virus in Hong Kong and its transmission to humans, and Hans Klenk (Marburg, Germany), who emphasized that, although outbreaks of both Marburg and Ebola infections have, to date, been self-limiting, our ignorance concerning the natural virus reservoir and the lack of immunoprophylactic and chemotherapeutic measures make these infections a matter of great biomedical concern. In fact, the chronology of filovirus infection during human epidemics and epizootics in non-human primates is, perhaps, prototypic of emerging/re-emerging viral pathogens.

Two further highlights of the meeting were the presentations by the Nobel-laureate Manfred Eigen (Göttingen, Germany) and the neuropathologist Adriano Aguzzi (Zürich, Switzerland). Professor Eigen explained the concept of sequence space, with its huge storage capacity and tremendous connectivity and demonstrated its validity with an experimental analysis of Qβ-phage RNA evolution in the face of ribonuclease selection pressure. Adriano Aguzzi’s presentation focused on the use of transgenic animal models to study the pathogenesis of prion (scrapie) infection in relation to the CNS compartment and the spread of prions to the CNS from peripheral sites. His essential conclusions were that the availability of endogenous PrPSc to the infectious agent, rather than the deposition of PrPSc, correlates with scrapie neurotoxicity in vivo, and that neuroinvasion from the periphery depends upon the neuroimmune interface. B-lymphocytes are crucial for neuroinvasion and they may provide a target for post-exposure prophylaxis.

Finally, Walter Doerfler (Köln, Germany) described a series of fascinating studies on the transmission of foreign DNA via the gastrointestinal tract or placenta in mammalian systems. He finds that foreign DNA ingested with the food supply can survive passage through the gastrointestinal tract and reaches peripheral white blood cells, spleen and liver via the intestinal epithelia and cells in the Peyer’s patches of the intestinal wall. When DNA is fed to pregnant mice, it can subsequently be detected in cells in various organs of new-born mice. The possible implications of these findings need to be further investigated.

Two biographies

Friedrich Loeffler was born on 24 June 1852 in Frankfurt an der Oder, Germany. He studied medicine in Würzburg and Berlin and from 1880 to 1887 he was first assistant to Robert Koch, employed at the ‘Kaiserliche Gesundheitsamt’ and later at the Institute for Infectious Diseases in Berlin. In the autumn of 1888, he was offered a professorship at the newly established Chair for Hygiene in Greifswald and he remained in this post for 25 years. Despite considerable resistance, in 1910 he was able to establish an institute for FMD research on the Island of Riems on the Baltic Sea coast near Greifswald. This institute, which today bears his name and belongs to the Federal Research Centre for Virus Diseases of Animals, was the first established institute of animal virology in the world. In the autumn of 1913, Loeffler was offered the position of Director at the Institute for Infectious Diseases in Berlin, the place at which his mentor, Robert Koch, had worked so successfully for many years. He died in Berlin in 1915. The discovery of the causative agents of diphtheria and FMD are sufficient to make Loeffler’s name immortal. Further important discoveries, such as the causative agent of glanders – a severely infectious disease of horses – and the causative agent of swine erysipelas – an earlier much-leared contagious disease, as well as the causative agent of mouse typhus, which he called Bacillus typhimurium, can also be accredited to him.

Paul Frosch was born in Jüterbog, Germany in 1860. In 1891, he received a position as assistant at the Institute for Infectious Diseases in Berlin, where he very soon became one of Robert Koch’s closest co-workers. In 1899, he became head of the scientific department at this Institute and from 1908
onwards he held a Professorship at the Chair for Hygiene at the Veterinary School in Berlin. He died in 1928 in Berlin.

References
