Construction and application of virus-like particles (VLPs) to monitor and prevent certain RNA virus infections

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In recent years, the intensification of virology research has led to the discovery of over thirty new pathogens responsible for infectious diseases. The majority of newly described etiological agents are RNA viruses, such as hepatitis C virus (HCV), human immunodeficiency virus (HIV), acute gastroenteritis virus (hNoV) (human norovirus), zoonotic influenza virus strains (H5N1, H7N9 strains), and SARS-CoV-2.

The development of molecular biology, genetic engineering, bioinformatics, and nanotechnology has allowed for the creation of alternative methods for studying dangerous pathogens. Virus-like particles (VLPs) are commonly employed as antigens in contemporary prophylactic preparations designed to prevent viral infections. Developing virus-like particles that mimic native viruses has become a crucial area of fundamental research. Obtaining virus-like particles for RNA viruses makes it possible to comprehend the molecular biology and replication cycle of these pathogens, which contributes to the development of safer, more effective vaccines and novel diagnostic tools. The basis for the prevention and surveillance of diseases not only in humans, but also in animals, is the use of protective agents and sensitive diagnostic tests.

In Poland, epidemics generated by RNA viruses, such as the avian influenza virus and the rabbit calicivirus, have been linked to high economic losses. Monitoring and preventing infections caused by these etiological agents would substantially decrease the economic losses associated with poultry and rabbit farming. The doctoral thesis describes the development of methods for monitoring and preventing infections induced by certain RNA viruses in Poland that cause significant economic losses. The primary objective of the research was the construction and characterization of virus-like particles (VLPs) obtained for Polish environmental strains of RNA viruses and their application in the development of vaccines and diagnostic tests. Two models of virus-like particles were tested in this study: orthomyxovirus (influenza A virus H5N1) (IVA/H5N1) and calicivirus (rabbit haemorrhagic disease virus) (RHDV). The selected research models represent two types of virus-like particles: icosahedral biological nanostructures composed of a single capsid protein for rabbit haemorrhagic fever virus and complex virus-like particles with a lipid envelope and several structural proteins for influenza type A virus. Real epidemiological concerns associated with the outbreak of H5N1 influenza virus in Poland and the spread of rabbit fever due to infection with rabbit calicivirus (RHDV.1) in Europe served as the basis for the selection of the presented research models. Polish viral strains of both pathogens were used to construct and characterize virus-like particles in order to develop diagnostic and preventative treatments.

The first model for scientific research was the orthomyxovirus IVA/H5N1. The purpose of the doctoral dissertation research was to characterize a Polish wild isolate of a highly pathogenic strain of influenza A H5N1 virus, as well as construct and develop diagnostic assays and protective treatments for poultry. In this research, domestic H5N1 influenza virus strains isolated in 2006 were utilized. The influenza virus genetic material was obtained from the National Veterinary Institute in Pulawy in the form of RNA. Individual gene sequences (ha, na, and m1) were sequenced, and the Polish isolate was then characterized using in silico analyses. The following were obtained as part of this project: (i) influenza A virus H5N1 antigens produced in a baculovirus system (haemagglutinin - HA, neuraminidase - NA, core protein - M1 and a shortened form of hemagglutinin stem - HA stalk); (ii) rabbit polyclonal sera obtained by immunization of rabbits with purified NA and HA surface glycoproteins. In addition, research has demonstrated that the expression of genes encoding structural proteins (HA, NA, and M1) in insect cells results in the formation of biological nanostructures known as virus-like particles. Insect cells were used to produce three types of virus-like particles derived from the H5N1 influenza virus: H5N1-HA VLPs containing the major surface glycoprotein HA, H5N1-HA/M1 VLPs containing the HA glycoprotein and the M1 core protein, and H5N1-NA/HA/M1 VLPs containing the HA glycoprotein, NA, and the M1 core protein. Evaluation of the immunogenic properties of the obtained nanostructures revealed that vaccination of chickens with H5N1-HA VLPs, H5N1-HA/M1 VLPs, and H5N1-HA/NA/M1 VLPs stimulated the immune system and led to the formation of neutralizing antibodies. The purified virus-like particles can serve as reference antigens for serological tests, including hemagglutination inhibition (HI) and ELISA assays. Furthermore, the obtained sera and antigens in the form of VLPs are valuable tools for diagnosis.

In addition, the objective of this research was to generate highly conserved universal influenza virus antigens in the form of hemagglutinin protein without the variable head region. The expression of a truncated form of the hemagglutinin gene in the form of a HA stalk containing a highly conserved LAH region (long alpha helix) confirmed the universal nature of the produced protein. The obtained results suggest that vaccination of hens with H5N1-HA/NA/M1 VLPs results in the formation of a pool of universal antibodies that recognize conserved epitopes located in the stalk region of various hemagglutinins in the form of a HA stalk.

The work performed for this doctoral dissertation was an integral part of the implementation project designated "Centre for the Biotechnology of Medicinal Products." The POIG.01.01.02-14-007/08-00 contract was for the development of a vaccine against highly pathogenic avian influenza.

Rabbit calicivirus was the second research model. In the present research, the Polish strain (SGM strain) of the RHD virus was analyzed. Molecular characterization of the virus strain revealed that the production of the main capsid protein (VP60 protein) in insect cells leads to the self-assembly of virus-like particles (VLPs-RHDV) that are morphologically similar to the native virus. The effectiveness and practicability of using VLPs-RHDV as a potential prophylactic vaccine for rabbits have been demonstrated, and diagnostic assays for monitoring RHD virus infections based on the virus-like particles have been developed. In addition, the long-term stability of VLPs-RHDV virus-like particles was examined and demonstrated in this study. This doctoral dissertation also contributed to KBN project 3P04B01923 entitled "Expression of virus-like particles of rabbit calicivirus and construction of a potential subunit vaccine against viral haemorrhagic disease of rabbits."

Using virus-like particles as antigens in the development of animal vaccines of the next generation is reasonable, according to the findings of this dissertation. In addition, the obtained research tools, such as serums and antigens, can be implemented commercially. The findings that were described provided new and valuable information that could contribute to the design of effective protective preparations against both influenza A virus and RHD virus.