Establish the virus like particle vaccine technology and rapid diagnostic kit platforms

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Disease rapid diagnostic kit and vaccines are very important for animals healthy. In our research, we establish the rapid diagnostic kit and virus like particle vaccine platforms for animal health.

Vaccination is considered the most cost-effective way to control pathogens and prevent diseases both in human and veterinary field. Currently, the majority of licensed vaccines are either live attenuated or killed, developed using conventional technologies. However, new subunit vaccines are getting a foothold in the veterinary vaccinology, and among these, virus-like particles (VLPs) represent one of the most appealing approaches. VLP vaccines combine many of the advantages of whole-virus vaccines and recombinant subunit vaccines, integrating key features that underlay their immunogenicity, safety and protective potential: (1) preservation of native antigenic conformation, (2) safety, as they are absolutely non-infectious and nonreplicating candidates, (3) higher stability than soluble antigens in extreme environmental conditions, (4) self-adjuvanting immunogenic delivery systems. Moreover, we establish the VLP vaccine platform for chicken Infectious bursal disease (IBD) vaccine development. IBD has been a great concern for the poultry industry for a long time, but particularly for the past decade. The target organ of IBDV is the bursa of Fabricius at its maximum development, which is a specific source for B lymphocytes in avian species. After oral infection or inhalation, the virus replicates primarily in the lymphocytes and macrophages of the gut-associated tissues. Then virus travels to the bursa via the blood stream, where replication will occur and caused the immunosuppression of chicken. The economic impact of both clinical and sub-clinical diseases warrants the search for and the use of efficient vaccines. Classical inactivated and subunit vaccines can not induce high and long-term antibody and T cell immune response. On the other hand, live vaccines proportional risks of reversion to virulence. Moreover, the VLP vaccine for IBDV is the best solution for IBDV infection in chicken.

Electron microscopy analysis of the recombinant protein revealed the presence of empty virus-like particles (Fig. 1A and B). These results demonstrated that IBDV capsid subunit proteins expressed in insect cells can be processed and co-assemble to form VLPs. For immunization studies, IBDV VLPs were partially purified from infected Sf9 cells and a control antigen was prepared from uninfected Sf9 cells. To determine the immunogenicity, chickens wer i.p. administered with 0.13 and 0.27 ug of IBDV VLP at days 3. Another group of chickens were injected with the control antigen with the same procedure. ELISA analysis showed that the control sera did not exhibit significant reactivity with the recombinant IBDV capsid subunit proteins (Fig. 2); in contrast, the anti-VLP sera reacted strongly with capsid antigens (Fig. 2). The chickens were challenged with the lethal dose of IBDV. The survival rates were 50% and 100% for the PBS and VLP vaccinated groups, respectively (Fig. 3). In contrast, all of the VLP vaccinated chickens remained healthy and survived throughout the course. In conclusion, the presented data convincingly show that recombinant IBDV-VLPs elicit high-titer antibodies to protect chiaken from lethal IBDV challenge, thus demonstrating the potential of IBDV-VLPs as a

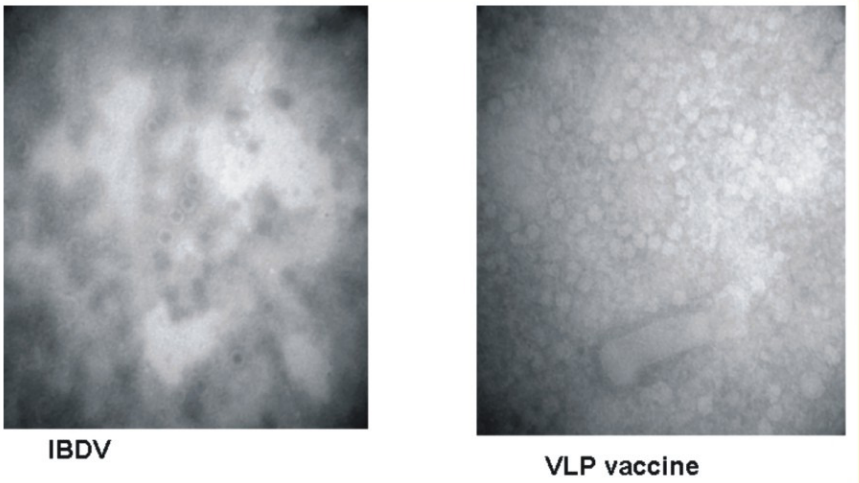

Fig1. Assembly of IBDV VLPs. Electron microscopy of IBDV (A) and IBDV-VLPs (B).

promising IBDV vaccine candidate. In the present study, we have established the VLP production platform for vaccine development. The technique platform can be used for safety, effective and cheap animal vaccines development.

On the other hand, the rapid detection of virus infection is essential for pre-vaccination and prompt identification of the spread of an outbreak. We are currently developing a rapid diagnostic kit for detection of many virus diseases. This kit is based on a chromatographic strip test and can be used in the field by technicians. The virus antigen can be detected from a drop of blood, serum sample or swap from suspected animals in just a few minutes. The colloidal gold test strip is a new immunochromatographic technique in which a cellulose membrane is used as the carrier, and a colloidal gold-labeled antigen or antibody is used as the tracer. This technology has several advantages over traditional immunoassays, such as simplicity of procedure, rapid operation, immediate results, low cost, and no requirements for skilled technicians or expensive equipment. Because of these characteristics, the colloidal gold test strip is suitable for the on-site detection of antibodies or antigens. For the detection of the viral antigen, specific monoclonal antibodies and chromatographic technology production platforms have been established in our laboratory. 1x106 copy of the virus was serially diluted, and 50 μl of each diluted sample was added to test strips. The band appeared at the test line with all dilutions less than 10 copies, but no band appeared when the dilution was 1or 0 copy.

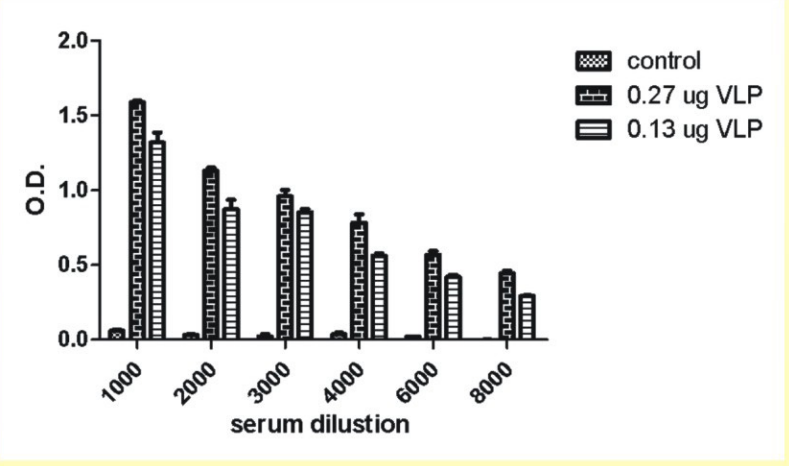

Fig 2. Antibody responses elicited by IBDV-VLP immunization. Chickens were injected intraperitoneally (i.p.) at days 3: IBDV-VLP equivalent to 0.13 and 0.27 μg of capsid protein,or the control lysate similarly prepared from uninfected Sf9 cells. The immunized chickens were sacrificed at week 6, and the serum and samples were collected and assayed. Results are representative of 2 independent experiments. Binding activity of the antisera to inactivated IBDV capside protein. The antisera were diluted 1:1,000 to 1:8,000.

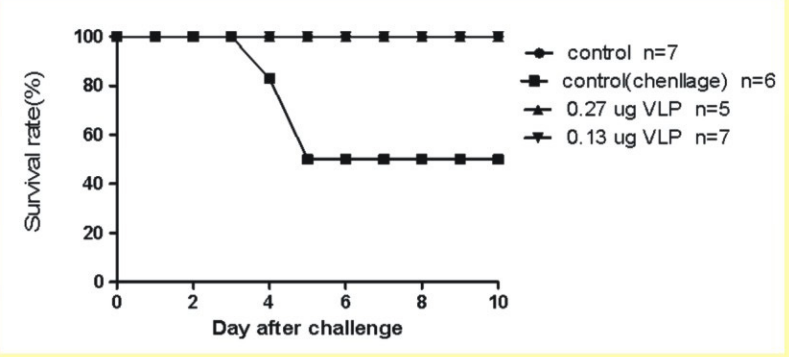

Fig 3. The IBDV VLP immunized chicken conferred protection against lethal IBDV challenge.

All of these results suggest that the rapid diagnostic kit platform and test strip prepared in the current study could be used in the specific diagnosis and epidemiological investigation We hope we can combine the rapid diagnostic and VLP vaccine production platforms to improve the animal health.

Fig 4. Sensitivity of the immune colloidal gold test strip.1x106 (line1), 1x105(line 2), 1x104(line 3), 1x103(line 4), 1x102 (line 5), 10(line 6), 1(line 7) and 0(line8) copy of virus were simultaneously tested by this strip test

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