

# Recombination of Goose Parvovirus VP3 Gene and Goose Interferon $\gamma$ Gene from Fowlpox Virus Immune Protection and Its Mechanism

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**Abstract:** Gosling plague (GP) is caused by goose parvovirus (GPV) gosling acute, subacute, and septic infectious diseases, commonly known as gosling plague. At present, the prevention and control of GP at home and abroad mainly adopts the method of immunizing female geese with attenuated vaccine. Goslings can be immunized, but there are still the phenomenon of dispersive virus and virulence reverse. It, therefore, is urgent to develop a new safe and effective vaccine. The recombinant avian pox virus vaccines rFPV-GoIFN $\gamma$ , rFPV-GPV-VP3 and rFPV-GoIFN $\gamma$ -VP3 were used to monitor their antibody levels and evaluate the protective mechanism of the vaccine in this study. This study lays a foundation for further *in vivo* testing of recombinant avian pox virus vaccine for goose parvovirus and provides a reference for the development of recombinant avian pox virus vaccine for other avian infectious diseases.

## 1 INTRODUCTION

cute, subacute, and septic infectious diseases of goslings are brought on by goose parvovirus and have a high incidence and mortality (Fang 1962; Fang 1981). There is no cure for the disease once it manifests clinically, which poses a severe threat to the industry's further growth (Soliman, Erfan 2020). As a result, there is an urgent need for an effective vaccine to stop the spread of this disease. Traditional vaccinations may pose risks like virulence recurrence and inactivation. Vaccines made using genetic engineering make up for these shortcomings. Numerous studies have demonstrated that the avian pox virus live vector vaccine can induce long-lasting humoral and cellular immunity in birds by continually expressing exogenous protective antigen genes.

Interferon (IFN) is a secreted multifunctional sugar egg white produced by host cells under the action of viruses or specific bioinducers. It has broad-spectrum antiviral, anti-tumor, and immunological regulation effects and is one of the most critical cytokines. In 1995, Lowenthal *et al.* (Lowenthal, Digby 1995) obtained IFN from chicken spleen lymphocytes, similar to mammalian interferon  $\gamma$  (IFN- $\gamma$ ) function. Digby *et al.* received IFN- $\gamma$  gene from induced T cells by RT-PCR. In 1999, Lambrecht *et al.* (Lambrecht, Gonze 1999) expressed IFN-I and IFN-II, respectively, and conducted activity studies, indicating that IFN expressed by *E. coli* and COS cells has antiviral and immunomodulatory effects as well as natural IFN. Moreover, the immunomodulatory ability of type II IFN- $\gamma$  interferon is more vital than that of IFN-I.

Interferon IFN has a strong immunomodulatory effect. IFN was discovered by Alickssacs *et al.* in 1957 when they infected chicken embryo chorioallantoic membrane with viral interference. Since then, its antiviral, anti-tumor, and regulatory immune response effects have attracted much attention from researchers. It has made outstanding achievements in the mechanism of action and *in vitro* recombinant expression. Many studies have shown that IFN $\gamma$  mainly inhibits the expression of tumor genes. In a tumor, IFN can control the immune system and reshape cancer through immune editing (Dunn, Koebel 2006). IFN- $\gamma$  is mainly involved in immune surveillance; Shreiber RD (Bui and Schreiber 2007) confirmed that IFN- $\gamma$  plays a significant role in specific immune-mediated immune balance. The antiviral effect of interferon is not to inactivate the virus but to block viral replication by binding interferon to cell surface receptors that activate specific genes to produce proteins. In 1998, Shi Wenfang's (Shi, 1998) research results explained that the antiviral effect of IFN- $\gamma$  was particular to the species. In addition, IFN- $\gamma$  has been shown to be effective against the Newcastle disease virus, avian influenza virus, and infectious bronchitis.

Presently, the prevention and treatment of GP at home and abroad mainly adopt the method of immunizing mother geese with the attenuated vaccine and immunizing goslings. Although it plays an essential role in the prevention of this disease, the attenuated vaccine has the phenomenon of dispersing virus and anti-virulence, which limits its application. Therefore, a safe and effective new vaccine is urgently needed. The recombinant poxvirus vaccine has attracted the attention of many researchers for its safety, stability, and ability to express exogenous genes, especially the fowlpox virus (FPV), which contains non-

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essential regions for replication, can insert exogenous genes to construct a polyvalent vaccine, and can continuously express exogenous antigens to stimulate the body to produce specific antibodies (Glávits, Zolnai 2005). And it is popularized and applied. However, the recombinant avian pox virus vector has specific toxic and side effects, leading to weight gain and suppression of the immune response in immunized animals. Different vaccines of the same vector will interfere with each other in the use process. In contrast, gamma interferon regulates the immune system and can also reduce the influence of the vector on the weight gain of immunized animals.

There is currently no research on creating a recombinant avipoxvirus vaccine using the goose IFN gene and the goose parvovirus protective antigen gene. The protective antigen gene in this study was the VP3 gene of goose parvovirus, which can promote the formation of neutralizing antibodies. The regulatory gene was the IFN gene. The two genes were combined to create the single expression and co-expression recombinant avian pox virus vaccines. Each vaccine's impact on immunological protection was assessed, and immune and challenge tests shed light on the immune system's workings. The results of this study will serve as a theoretical foundation for creating a novel vaccination to prevent goose parvovirus disease.

## 2 Materials and methods

Three kinds of recombinant avipoxviruses containing exogenous target genes namely, rFPV-GPV-VP3, rFPV-GoIFN $\gamma$  and rFPV-GoIFN $\gamma$ -VP3, were prepared by molecular cloning technology and recombinant virus rescue technology. Based on detecting the reactivity of recombinant avipoxviruses expressing exogenous proteins, antiviral titer and standard virus virulence, Gosling inoculation and challenge tests were conducted. The control live vaccine was goose parvovirus vaccine (SYG41-50, Sinophinherb Group Yangzhou Weike Biological Engineering Co., LTD.). Experimental animal grouping: A total of 105 2-d-old goslings were randomly divided into 7 groups with 15 goslings per group. Group 1 (S-FPV-017 group); Group 2 (rFPV-GPV-VP3 group); Group 3 (rFPV-GoIFN $\gamma$ -VP3 group); Group 4 (rFPV-GoIFN $\gamma$  group); Group 5 (SYG41-50 strain group); Group 6 (rFPV-GoIFN $\gamma$ + SYG41-50 strain group). In the seventh group (PBS control group), the vaccine was inoculated in the neck subcutaneous place without blood vessels. After 3 weeks of immunization, the vaccine was challenged with virulent GPV strain (YBLJ strain). The humoral and cellular immunity levels were detected by using cloacal swabs and blood of goslings at different stages.

## 3 Results and discussion

Since the mid-1970s, the rapid development of molecular biology technology has made it possible to develop and apply live gene vector vaccines. Live virus vectors not only lose pathogenicity but also can continue to express foreign protective antigen genes in the immune body, which has the advantages of both inactivated and live vaccines. Among many live virus vectors, avian pox virus (FPV) vectors are the most mature in avian disease prevention, which can stimulate the body to produce humoral and cellular immune responses, and can continue to replicate in poultry after immunization, with a long period of resistant protection (Lousberg, Diener 2011). However, the avian pox virus vector vaccine has a specific residual toxicity, which has a particular inhibitory effect on the weight growth of chicks. To avoid interference between vaccine vectors during the use process, researchers have tried various research methods to reduce the toxic and side effects of FPV, that is, to introduce cytokines into the vaccine. Yunfeng Wang *et al.* (Wang 2005) constructed recombinant fowlpox virus co-expressing IBV-S1 and  $\gamma$  interferon (IFN) genes by inserting  $\gamma$  interferon into the recombinant vaccine. Rautenschlein *et al.* (Rautenschlein, Sharma 1999) cloned chicken IFN $\gamma$  gene and protective antigen gene into an avian poxvirus vector and overcame this problem through co-expression. The experimental results show that compared with a single expression of protective antigen, co-expression improves immune efficacy and decreases the vector's toxic and side effects. The results showed that the inhibition of recombinant avipoxviruses on the weight growth of chicks was reduced. With the development of genetic engineering, genetically engineered vaccines have gradually become a research hotspot. Some recombinant fowlpox viruses have been commercialized. For example, the recombinant fowlpox virus vaccines expressing the gB gene of infectious Laryngotracheitis virus (LTV) have been approved by the Ministry of Agriculture of China and started commercial production (Tong, Zhang 2001).

In previous experiments, we verified the stable expression of the GPV-VP3 gene and goose IF $\gamma$  gene in recombinant avian pox virus. Indirect immunofluorescence assay (IFA) was used to demonstrate further the expression of foreign genes and the reactivity of recombinant proteins. The preliminary IFA results showed that mouse monoclonal antibody against GPV-VP3 protein and mouse polyclonal antibody against goose IFN- $\gamma$  protein prepared previously were used as the primary antibody, and sheep anti-mouse IgG-FITC was used as the secondary antibody. Specific green fluorescence could be seen in both detection holes under the fluorescence microscope. It was further indicated that RFPV-GoIFn-gamma-VP3 recombinant virus could stably express GPV-VP3 recombinant protein and goose IFN- $\gamma$  protein in CEF (Song 2017; Wang, Zhang 2018).

Recombinant avian pox vaccine research is still lacking domestically and internationally for the GPV-VP3 and goose IFN $\gamma$  genes. There is much room for improvement in the recombinant avian pox virus vector vaccine with GPV, which also has some practical value

(Zhang, Han 2018). Therefore, the goose IFN $\gamma$  gene was employed as the regulatory gene in this study. The GPV-VP3 gene was used as the protective antigen gene to build a recombinant avian pox virus with gene co-expression successfully. *In vivo* immunization and attack investigations were also carried out. The results showed that on the 9th day after vaccination, GPV antibody was positive in all groups. The antibody level of attenuated vaccine strain SYG41-50 was the highest, while the antibody level of the rFPV-GoIFN $\gamma$ VP3 group was the lowest. On the 6th day after the GPV challenge, GPV could not be detected in four groups: rFPV-GPVVP3, rFPV-GOIFN $\gamma$  VP3, SYG41-50, and rFPV-GoIFN $\gamma$ +SYG41-50. The levels of CD4+ and CD8+ in the rFPV-GoIFN $\gamma$ VP3 group increased the expression of CD8+ after immunization, while the levels of CD4+ and CD8+ in the other groups were stable. The rFPV-GoIFN $\gamma$ -VP3 group and the attenuated vaccine strain SYG41-50 group obtained immune protection in 14/15 cases, which was second only to the rFPV-GoIFN $\gamma$  +SYG41-50 strain group (15/15). The protection rate of the single immunization rFPV-GPV-VP3 group was 13/15. These results suggested that GoIFN $\gamma$  promoted the expression of CD8+, increasing the cellular immunity level of the immune body and making up for the low antibody level of the rFPV-GoIFN $\gamma$ -VP3 group. It, therefore, finally had the same protection as the attenuated vaccine strain SYG41-50. rFPV-GOIFN $\gamma$ -VP3 live vector vaccine is the best candidate to improve vaccine safety and avoid the potential risk of scatter virus.

## 4 Conclusion

The best potential vaccination in this investigation was the rFPV-GoIFN $\gamma$ -VP3 recombinant live vector avian pox virus vaccine after seven different inoculation tests were carried out on attacking geese.

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