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Comparison of the immune responses in BALB/c mice following immunization with DNA-based and live attenuated vaccines delivered via different routes

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ABSTRACT

The objective of this study was to compare immune responses induced in BALB/c mice following immunization with pcDNA-GPV-VP2 DNA by gene gun bombardment (6 μg) or by intramuscular (im) injection (100 μg) with the responses to live attenuated vaccine by im injection (100 μl). pcDNA3.1 (+) and physiological saline were used as controls. Peripheral blood samples were collected at 3, 7, 14, 21, 28, 35, 49, 63, 77 and 105 d after immunization. T lymphocyte proliferation was analyzed by MTT assay and enumeration of $CD4^+$, and $CD8^+$ T cell populations in peripheral blood was performed by flow cytometric analysis. Indirect ELISA was used to detect IgG levels. Cellular and humoral responses were induced by pcDNA-GPV-VP2 DNA and live virus vaccines. No differences were observed in T cell proliferation and $CD8^+$ T cell responses induced by the genetic vaccine regardless of the route of delivery. However, $CD4^+$ T cell responses and humoral immunity were enhanced in following gene gun immunization compared with im injection of the genetic vaccine. Cellular and humoral immunity was enhanced in following gene gun delivery of the genetic vaccine compared with the live attenuated vaccine. In conclusion, the pcDNA-GPV-VP2 DNA vaccine induced enhanced cellular and humoral immunity compared with that induced by the live attenuated vaccine.

1. Introduction

Goose parvovirus (GPV) infection, also known as Derzsy's disease, goose hepatitis, infectious myocarditis or 'goose plague', is a highly contagious disease affecting domestic goslings and Muscovy ducklings [1]. This type of autonomous parvovirus replicates in the nucleus of erythroid precursor cells and predominantly disrupts the digestive, urinary and immune systems [2]. Diarrhea and fibrous hemorrhagic necrotic enteritis are the main clinical and pathological manifestations of GPV. A live attenuated vaccine is currently used to control this disease because conventional vaccines induce deficient cellular immune responses with limited ability to counteract GPV infections. It can be speculated that the virulence of the live attenuated vaccine will be efficacious in the control of this infection in geese [3,4]. Therefore, the development of a safe and effective vaccine represents a significant focus of research. The right open reading frame (RORF) of GPV encodes three classes of structural proteins: VP1, VP2 and VP3 [3]. To date, to the best of our knowledge, no published reports describing the immunogenicity of pcDNA-GPV-VP2 have been published.

Genetic vaccines generate $CD4^+$ and $CD8^+$ T cell responses that are similar to those induced by viral infection and induce humoral immunity simultaneously. Vaccines based on this modality are dependent on two major mechanisms:(a) the persistence of memory B and T cells stimulated at time of vaccination/infection and (b) the persistence of long-lived plasma cells (termed "memory effector B cells"), which continue to produce antibody for years after initial immune stimulation [5]. Therefore, genetic vaccines play an important role in resisting or attenuating viral infections. Furthermore, genetic vaccines provide prolonged antigen expression, leading to amplification of immune responses and induction of memory responses against infectious agents [6]. The gene gun represents a highly effective method for tissue transfection. When the epidermis is transfected, expression of DNA encoded antigens is subject to immune surveillance by the skin-associated lymphoid tissue. This lymphoid tissue is rich in cells (such as epidermal Langerhans cells) that are capable of presenting transfected antigens to the T-helper component of the immune system [7].

In this study, the pcDNA-GPV-VP2 plasmid constructed in our laboratory was used to immunize BALB/c mice via different routes and the responses generated were compared with those induced by a live attenuated viral vaccine. Analysis of T cell proliferation (MTT assay), enumeration of $CD4^+$, and $CD8^+$ T cell populations in peripheral blood (flow cytometry) and determination of IgG

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levels (indirect ELISA) were performed to investigate the differences between the immune responses generated by the genetic vaccine and the live attenuated vaccine and the underlying mechanisms of these GPV-specific responses.

2. Materials and methods

2.1. Plasmid and vaccine

Vector pcDNA3.1 (+) was purchased from Invitrogen. The pcDNA-GPV-VP2 plasmid was constructed in our laboratory. The plasmid was prepared and purified by alkaline lysis and PEG-$MgCl_2$ precipitation. The live attenuated GPV vaccine was prepared in our laboratory [8].

2.2. Immunogenicity

BALB/c mice obtained from the Laboratory Animal Centre of Guangzhou Medical University were shown to be negative for GPV-specific antibodies. Mice were allocated to five groups ($n = 28$ per group) as follows: (A) gene gun delivery of pcDNA-GPV-VP2 DNA genetic vaccine (6 μg/mouse); (B) im injection of pcDNA-GPV-VP2 genetic vaccine (100 μg/mouse); (C) im injection of live attenuated vaccine (100 μl/mouse); (D) control im injection of pcDNA3.1 (+) (100 μg/mouse); and (E) control im injection of physiological saline (100 μl/mouse).

Peripheral blood samples were collected at 3, 7, 14, 21, 28, 35, 49, 63, 77 and 105 d after immunization for analysis of T lymphocyte proliferation, $CD4^+$, and $CD8^+$ T enumeration. Serum samples collected at 133, 161, 189 and 217 d were used to determine IgG levels [8].

2.3. T lymphocyte proliferation assays

Blood was collected from individual mice into a sterilized assembly containing 2% Heparin Sodium in a ratio of 10:1. Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation. Cells were resuspended in sterile PBS, washed three times and resuspended in RPMI1640 (GIBCO BRL) growth medium at 5×10^6 cell/ml. Cells were added into flat-bottomed 96 well-culture plates in triplicate (100 μl/well). All wells were pulsed with Concanavalin A (ConA) (Sigma, USA) at a concentration of 25 μg/ml per well and RPMI was used in positive and unstimulated controls. Plates were incubated at 37 °C under 5% CO_2 for 68 h. MTT (50 μg/well) was added to each well for the final 4 h of the incubation period. Subsequently, 100 μl of dimethyl sulfoxide (10% SDS–0.04 M HCl) was added to each well, mixed for color development and incubated at 37 °C for 4 h. The optical density was measured at 490 nm on an ELISA Plate Reader (ELx800, Bio- tek).

2.4. T lymphocyte subgroup assays

Blood samples (50 μl) were lyzed by the addition of fresh ammonium chloride solution (500 μl). Samples were incubated for 10 min at room temperature, centrifuged for 5 min at 1000 rpm, washed and resuspended in 1 ml PBS. Detection antibodies (1 μl), FITC-labeled anti-mouse $CD4^+$ and $CD8^+$ antibodies (BD Biosciences, Nos.: 757454 and 753030, respectively) were added and incubated for 30 min at 8 °C. Cells were washed with PBS and 10,000 cells were assayed by flow cytometry using a fluorescence-activated cell sorter (BD FACS Calibur).

2.5. GVP-specific antibody assays

GPV was propagated in embryonated duck eggs. Allantoic fluid was collected and subjected to two rounds of a freezing at −20 °C and thawing followed by centrifugation at 2500 rpm for 25 min. The virus contained within the liquid phase was purified three times by 40% CsCl gradient centrifugation (2500–3000 rpm for 30 min, 8000–10,000 rpm for 50 min and 10,000 rpm for 60 min) and harvested by ultracentrifugation. The pellet was resuspended in PBS.

Anti-GPV antibodies were measured by indirect ELISA. Polystyrene plates were coated overnight at 4 °C with GPV (100 μl/well) at a final concentration of 5 g/ml. Plates were washed with PBS containing 0.1% Tween 20 (PBST) and blocked for 2 h at 37 °C with 100 μl of blocking buffer (PBS containing 1% BSA). Plates were washed with PBST and threefold serial dilutions of individual serum samples (92 μl), were added starting at a 1 in 200 dilution in buffer (PBST with 0.1% BSA) and incubated for 30 min at 37 °C. Serum from mice immunized with physiological saline was used as a negative control. After three washing cycles, plates were incubated with a 10,000-fold dilution of peroxidase-conjugated Affinipure Goat Anti-Mouse IgG (H + L) (No.: ZB-3451, Zhong-Shan Golden Bridge Biotechnology Co. Ltd., Beijing, China) for 1 h at 37 °C. After the plates were washed, excess reagent was removed. Peroxidase activity was measured by addition of TMB (No.: 4533B56A, MRESCO) in citrate-phosphate buffer (0.2 M Na_2HPO_4, 0.1 M citric acid, pH 5.0) and 30% H_2O_2 at the ratio of 1000:1 The reaction was stopped after 10 min by addition of 2 M H_2SO_4 and absorbance measured at 450 nm.

2.6. Statistical analysis

All statistical analyses were performed using the SPSS 12.0 software for Windows. A two-tailed Student's *t*-test was used to compare the differences in mean values between groups. *P*-values less than 0.05 were deemed to be statistically significant.

3. Results

MTT assays showed that that non-specific T cell proliferation was induced following immunization with the pcDNA-GPV-VP2 genetic vaccine by gene gun bombardment and im injection ($P < 0.01$ and $P < 0.05$, respectively). Proliferation increased from 14 d, reached a peak at 28 d and was subsequently reduced. Similar proliferation was stimulated by immunization with the genetic vaccine delivered by gene gun bombardment (6 μg) or im injection (100 μg). 6 μg group was different to others (except 100 μg group) at 7–105 d, and 100 μg group was different to others (except 6 μg group) at 21–35 d (Table 1).

The number of $CD4^+$ T cells increased from 14 d, reached a peak at 28 d and was subsequently reduced. 6 μg group and 100 μg group

Table 1
Results of T lymphocyte proliferation test in BALB/c mice immunized with the gene vaccine or the attenuated vaccine.

Time (d)	A	B	C	D	E
	(Od_{490} value)				
3	0.27	0.30	0.32	0.39	0.35
7	0.52	0.42	0.38	0.41	0.37
14	0.56	0.48	0.40	0.42	0.39
21	0.62	0.64	0.48	0.49	0.41
28	0.81	0.88	0.51	0.43	0.43
35	0.77	0.72	0.48	0.44	0.42
49	0.54	0.43	0.44	0.46	0.40
63	0.51	0.39	0.40	0.49	0.39
77	0.47	0.35	0.34	0.39	0.35
105	0.39	0.36	0.33	0.38	0.36

Note: A: pcDNA-GPV-VP2 DNA vaccine (6 μg/mouse) delivered by gene gun injection; B: pcDNA-GPV-VP2 genetic vaccine (100 μg/mouse) delivered by intramuscular (im) injection; C: live attenuated vaccine (100 μl/mouse) delivered by im injection; D: control delivery of pcDNA3.1 (+) (100 μg/mouse) by im injection; E: control delivery of physiological saline (100 μl/mouse) by im injection.