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An Immunoenzyme Linked Assay (ELISA) for the Detection of Antibodies to Truncated Glycoprotein D (tgD) of Bovine Herpesvirus-1

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Abbreviations: BGH polyA, bovine growth hormone polyadenilation; BHV-1, bovine herpesvirus-1; ELISA, enzyme-linked immunosorbent assay; i.d., intradermally; i.m., intramuscularly; i.n., intranasally; p.c., post challenge; PCR, polymerase chain reaction; Plasmid pcDNA3.1-tgD, truncated form of gD into pcDNA3.1 Plasmid pET-23d; p.v., post vaccination; SN, serum neutralization; T0, the start of the experiment, time 0

INTRODUCTION

Bovine herpesvirus-1 (BHV-1) is responsible for a variety of clinical signs. It is widespread in cattle and causes severe economic losses (Castrucci *et al*., 2002a, b). To prevent the infection several live and inactivated vaccines are commonly used. However, due to their short-term immunity and incomplete protection, new vaccine strategies have been proposed such as genetic vaccination (Babiuk *et al*., 1999). With this aim a DNA vaccine, with a plasmid expressing the tgD glycoprotein, known to be responsible for the virus antigenicity and consequent immunogenicity (Castrucci *et al*., 2004; Gupta *et al*., 1998), has been investigated. In the present study, the ELISA reaction was performed in order to detect specific antibodies in calves vaccinated with a DNA vaccine using the pcDNA3.1-tgD plasmid.

MATERIALS AND METHODS

Plasmid pcDNA3.1-tgD was constructed by cloning the BHV-1 gene encoding tgD into pcDNA3.1 (Invitrogen, San Diego, CA) under the control of the Cytomegalovirus enhancer/early promotor and with a BGH polyA signal. The sequence for the tgD was obtained by PCR. Fifteen calves seronegative to BHV-1, subdivided into 5 groups, were used. Group 1 was inoculated i.m. with 500 μg of vaccine; Group 2 was inoculated i.d. with 250 μg of

vaccine and group 3 received 250 μg of vaccine by the i.n. route. Six calves (in groups 4 and 5) were controls. Seventy-nine days after the first injection of vaccine all calves were infected i.n. with $5 \times 10^{8.50}$ $TCID_{50}$ of virulent BHV-1. Serum samples, obtained at T0, at 42 days p.v., and at T0 and 14 days after challenge infection, were tested for antibody response by SN test and ELISA. Neutralization titres were expressed as $\log_2$ of the highest dilution inhibiting cytopathic effect.

Truncated gD was obtained from BHV-1 total DNA, followed by PCR product cloning using pET-23d recombinant vector. *E.coli* BL21pLysS was used for protein expression; protein concentration was evaluated according to the Bradford method (Bradford, 1976). The tgD concentration was determined using a reference immune serum to BHV-1 diluted 1:100; the anti-bovine IgG HRPO conjugated activity was tested with the same reference serum. The results showed that 0.85 μg/ml was the optimal tgD concentration, while the dilution of the conjugate was 1:500. Microtiter plates (Nunc Maxisorp) were coated with tgD, which was diluted in 0.05 M carbonate buffer, pH 9.6 at the above mentioned concentration (Dall'Ara and Re, 1993). After incubation overnight at 4°C, the plates were rinsed in PBS containing 0.05% Tween 20 (PBST) 3 times. The standard curve was determined by using two fold serial dilutions (from 1:100 to 1:12800) of reference and normal bovine serum, respectively. Serum samples diluted at the same concentration were then tested in duplicate. All plates were rinsed three times with PBST, incubated at 37°C for 1 hour, and after addition of anti-bovine IgG HRPO, were incubated for 1 hour at 37°C. The substrate solution was prepared by adding 60 mg of the chromogen *o*-Phenylenediamine dihydrochloride (OPD, Sigma), and 50 μl of 30% H_2O_2 to 10 ml of 0.05 M citric acid buffer, pH 4.5. The reaction was stopped after 10–15 min by adding 2N H_2SO_4 to each well. Readings were performed using a spectrophotometer with a 492 nm filter. For the end-point titre, a positive value was scored for any sample with an optical density (OD492) greater than three standard deviations above the background (calculated using 12 wells free of primary antibodies) and expressed as $\log_2$. All reagents were added in 100 μl volumes. Tests were repeated three times.

RESULTS

Neutralizing antibodies were detected on 42 days p.v. only for calves vaccinated by the i.m. route. After challenge exposure all calves seroconverted (14 days p.c.). There was no antibody production to BHV-1 in the vaccinated calves up to 21 days post vaccination. The antibody titre of these calves underwent a slight drop (0.40 $\log_2$) on the day of the challenge, after which the neutralization titre increased, reaching 5.00 $\log_2$ at 14 p.c. days. The other vaccinated calves (i.n. and i.d. routes) did not produce antibodies after vaccination and their sera showed neutralizing activity for the first time on day 14 p.c., with neutralization titres ranging from 4.00 $\log_2$ to 3.00 $\log_2$ for calves vaccinated i.d. or i.n., respectively. The plasmid vector did not induce neutralizing antibodies to BHV-1 in any of the calves inoculated i.m., i.d. or i.n. As far as ELISA test is concerned the results were generally analogous to those obtained with the neutralization test; in that the only calves which produced antibodies to BHV-1 were those vaccinated i.m., whose sera showed a titre of 4.90 $\log_2$ at 14 p.c. days. A seroconversion was observed in all animals after challenge exposure with values ranging

from 3.00 $\log_2$ to 4.90 $\log_2$. However, the highest titres were detected in the intramuscularly vaccinated group (mean value 4.90 $\log_2$).

DISCUSSION

To conclude, the ELISA test can permit detection of antibodies specific for the tgD of BHV-1 expressed from the plasmid pcDNA3.1-tgD. Because of this, DNA vaccination efficacy can be monitored using this technique, which is reproducible, simple and easily standardizable.

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REFERENCES

Babiuk, L.A., Lewis, J., Van Den Hurk, S. and Braun, R., 1999. DNA immunization: present and future. *Advances in Veterinary Science*, **41**, 163–179

Bradford, M.M., 1976. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytic Biochemistry*, **72**, 249–254

Castrucci, G., Frigeri, F., Salvatori, D., Ferrari, M., Sardonini, Q., Cassai, E., Lo Dico, M., Rotola, A. and Angelini, R., 2002a. Vaccination of calves against bovine herpesvirus-1. Assessment of the protective value of eight vaccines. *Comparative Immunology, Microbiology and Infectious Diseases*, **25**, 29–41

Castrucci, G., Frigeri, F., Salvatori, D., Ferrari, M., Lo Dico, M., Rotola, A., Sardonini, Q., Petrini, S. and Cassai, E., 2002b. A study on latency in calves by five vaccines against bovine herpesvirus-1 infection. *Comparative Immunology, Microbiology and Infectious Diseases*, **25**, 205–215

Castrucci, G., Ferrari, M., Marchini, C., Salvatori, D., Provinciali, M., Tosini, A., Petrini, S., Sardonini, Q., Lo Dico, M., Frigeri, F. and Amici, A., 2004. Immunization against bovine herpesvirus-1 infection. Preliminary tests in calves with a DNA vaccine. *Comparative Immunology, Microbiology and Infectious Diseases*, **27**, 171–179

Dall'Ara, P. and Re, G.N., 1993. La tecnica ELISA: protocollo di standardizzazione e validazione per la diagnosi e il monitoraggio di malattie infettive. *Bollettino di microbiologia e indagini di laboratorio*, **13**(6), 9–22

Gupta, P.K., Saini, M., Bandyopadhyay, S.K. and Garg, S.K., 1998. Bovine herpesvirus type 1 glycoprotein c expression in MDBK cell and its reactivity in enzime-linked immunosorbent assay. *Acta virologica*, **42**, 397–400

Kemey, D.M. and Challacombe, S.J., 1989. *ELISA and Other Solid Phase Immunoassays, Theoretical and Practical Aspects* (Ed. John Wiley and Sons, Chichester), 1–29