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Immune responses to non-structural protein 3 (NS3) of bovine viral diarrhoea virus (BVDV) in NS3 DNA vaccinated and naturally infected cattle

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Abstract

Immune responses to non-structural protein 3 (NS3) of bovine viral diarrhoea virus (BVDV) were investigated. cDNA encoding NS3 from type 1a BVDV was used to vaccinate five calves, another five calves remained unvaccinated. Three weeks after final vaccination animals were challenged intranasally with heterologous type 1a BVDV. Anti-NS3 antibodies were detected in only one animal post-vaccination. Partial protection from virus challenge was observed in the vaccinates. Virus was not isolated from nasal mucosa of two vaccinates, and virus clearance from nasal mucosa was faster in the vaccinates compared to the controls. While elevated rectal temperatures were evident in both groups 7 days post-challenge, the mean increase in the controls was twice that observed in the vaccinates. In conclusion, NS3 DNA vaccination induced humoral immunity in one calf, and prevented fever and virus establishment in the nasal mucosa in 2/5 calves, demonstrating the efficacy of NS3 vaccination, which may benefit future development of pestivirus and flavivirus vaccines.
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1. Introduction

Bovine viral diarrhoea virus (BVDV) is an economically important pathogen infecting cattle worldwide. BVDV is a pestivirus with a small-enveloped single-stranded positive sense RNA genome of approximately 12.5 kb. The large open reading frame gives rise to several structural and non-structural proteins. The most antigenic structural protein, E2, induces neutralising antibody in acute infection (Donis et al., 1988) and is an ideal candidate for a subunit vaccination strategy, although when administered as a single antigen, its highly variable sequence limits the cross-strain immunity induced. In the work by Bruschke et al. (1997), Harpin et al. (1999) and Nobiron et al. (2003), immunisation with a BVDV type 1 E2 induced only partial protection to heterologous virus challenge. The latter studies used a DNA vaccination regime as, in many systems, DNA vaccination has been shown to be effective in inducing protective immunity to endogenously expressed protein as reviewed in van Drunen Little-van den Hurk et al. (2001). To overcome the challenge posed by E2 sequence variability, the non-structural proteins, which are more conserved across BVDV virus strains (Deng and Brock, 1992), may assist in induction of cross-strain immunity.

The aim of this study was to assess whether DNA vaccination with the non-structural protein 3 (NS3) gene from a type 1a BVDV strain would induce protection against infection with a heterologous BVDV strain.

2. Materials and methods

2.1. Cells and viruses

BVDV free foetal bovine lung (FBL) cells were cultured in MEM containing Glutamax 1, Earles salts and 25 mM HEPES (Invitrogen) and 10% foetal bovine serum (PAA), 1% glutamine (Invitrogen) and 1% antibiotic/antimycotic (Invitrogen). The Ky1203nc type 1a strain (Clarke et al., 1987) and 456497 type 1a strain of BVDV (field isolate provided by the Veterinary Laboratories Agency) were propagated in FBL cells.

2.2. Plasmid

The gene encoding NS3 from strain Ky1203nc was cloned into pTriEx1.1Neo (Novagen) and purified from *Escherichia coli* XL1-blue (Stratagene) using anion-exchange resins (Qiagen). NS3 protein expression was confirmed in vitro after transient DNA transfection of FBL cells.

2.3. Vaccination and challenge

All animals were confirmed BVDV antibody free prior to inclusion in the study. Five calves were vaccinated intradermally with a total of 500 μg pTriExNS3 DNA in 1 ml 0.9% saline, on three occasions, 21 days apart. Five calves received no vaccination. Viral challenge ($5 \times 10^6 TCID_{50}$ BVDV strain 456497 per animal) was by the intranasal route 3

weeks after final vaccination. All animals were bled at each vaccination and on days 0, 3, 4, 5, 6, 7, 10, 14 and 21 following challenge. A vector plasmid-only group was not included in this study as Nobiron et al. (2003) confirmed that correlates of protection observed were due to the presence of the cloned gene and any non-specific stimulation of the immune system by the DNA vector had no effect on virus shedding, antibody responses, pyrexia or nasal secretions.

2.4. Virus isolation

Nasal swabs were taken on days 0, 3, 4, 5, 6, 7 and 10 following challenge. Samples were passaged twice in FBL cells and viable virus detected by immunofluoresence using BVDV hyperimmune serum and Cy3-labelled rabbit anti-bovine IgG (Stratech Scientific).

2.5. Clinical evaluation

Rectal temperatures were measured throughout challenge and animals were scored on dyspnoea, coughing, nasal discharge and demeanour. Scores ranged from 1 (normal) to 3 (high clinical severity).

2.6. ELISAs

Microtitre plates coated with mab WB112 to BVDV NS2-3 (Veterinary Laboratories Agency) were incubated with BVDV infected FBL cell lysate. Sera were added and monoclonal anti-bovine IgG clone BG18 biotin conjugated (Sigma) used as detecting antibody. Horseradish peroxidase (hrp)-conjugated Streptavidin (Amersham Biosciences) was added and the reaction visualised with *o*-phenylenediamine dihydrochloride (Sigma). Isotypes were determined using hrp-conjugated isotype specific antibodies (Bethyl).

3. Results

3.1. NS3 specific responses after intradermal vaccination

After a single vaccination, one out of five animals had seroconverted to NS3. The antibody titre in this animal continued to rise throughout the study and was at least 50% higher than the remaining animals 21 days post-challenge (Fig. 1A). NS3 specific antibodies were non-neutralising to homologous virus (data not shown), with an isotype of IgG1. The responding calf also showed an enhanced response to E2 21 days post-challenge compared to the other animals (data not shown).

3.2. Protection from challenge

The mean number of animals from which virus could be isolated was significantly higher in the unvaccinated calves on day 3 ($P = 0.040$), day 6 ($P = 0.004$) and day 7 ($P = 0.040$) compared to the vaccinates. Virus shedding from the nasal mucosa of two

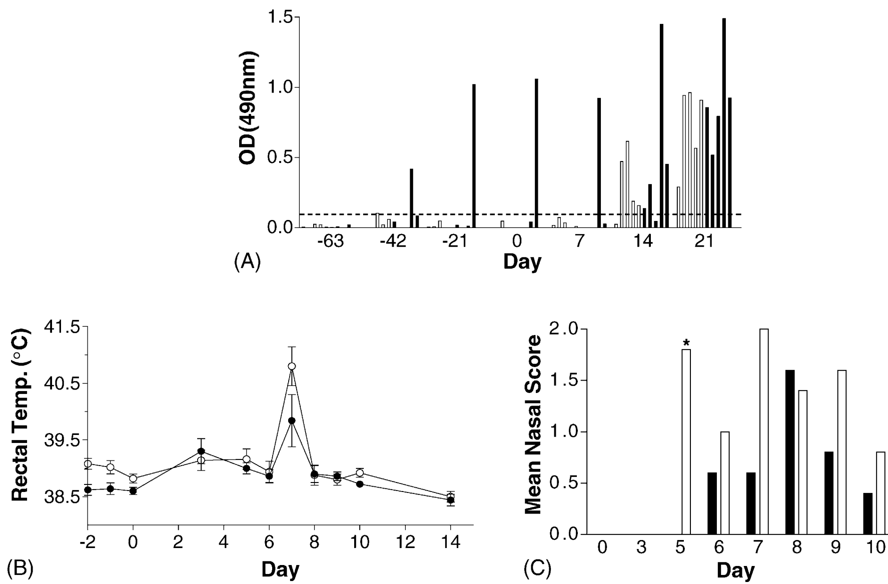

Fig. 1. Effect of vaccination with pTriExNS3. (A) Serum antibody responses in calves vaccinated three times with pTriExNS3 (■), unvaccinated (□) and subsequently challenged with BVDV. Vaccinations were on day −63, −42 and −21, challenge on day 0. Dotted line indicates ELISA negative cut off value. (B) Mean rectal temperature (error bars show S.E.M.). (C) Mean nasal discharge score ("*" indicates significant values) were recorded prior to and post-challenge, pTriExNS3 vaccinated (●/■) and unvaccinated (○/□).

vaccinated animals was completely prevented throughout the study and was only detected in 3/5 animals on a single day (5 or 6) (Table 1). In the control group, all animals had detectable virus from nasal mucosa between days 3 and 10 with 4/5 animals positive for 4 or more days.

Table 1
Virus isolation following challenge

Group	Calf number	Day post-challenge				
		3	5	6	7	10
NS3-vaccinated	5	–	–	–	–	–
	8	–	–	+	–	–
	9	–	+	–	–	–
	13	–	–	–	–	–
	21	–	+	–	–	–
Control	1	+	+	+	+	–
	3	–	–	+	–	–
	10	+	+	+	–	–
	15	+	+	+	+	–
	20	–	–	+	+	+

Virus isolation from nasal mucosa was performed by immunofluorescent assay after two passages in FBL cells.

Table 2
Rectal temperatures (°C) following challenge

Group	Calf number	Day								
		0	3	5	6	7	8	9	10	14
NS3-vaccinated	5	38.5	39.4	38.7	39.0	39.9	38.8	39.0	38.6	38.3
	8	38.5	38.9	39.2	38.9	38.8	38.5	38.9	38.8	38.5
	9	38.5	39.2	38.9	38.6	41.0	39.4	38.8	38.7	38.8
	13	38.6	38.9	39.0	38.6	38.8	39.0	38.6	38.8	38.4
	21	38.8	40.1	39.2	39.2	40.7	38.8	39.0	38.7	38.2
Control	1	38.8	38.8	39.3	38.7	41.2	38.5	38.7	39.2	38.3
	3	39.1	39.6	39.6	39.4	41.5	39.2	38.9	38.9	38.8
	10	38.8	39.1	39.4	39.3	39.5	39.2	38.8	38.9	38.6
	15	38.6	38.7	38.6	38.4	40.9	38.4	38.5	38.7	38.5
	20	38.8	39.5	38.9	38.9	40.9	39.1	39.1	38.9	38.3

Following challenge, an increase in the mean rectal temperature was observed on day 7 in both groups. The mean temperature of the vaccinates was only 0.5 °C above normal yet the mean temperature of the control group rose to 1.5 °C above normal (Fig. 1B), although this was not statistically significant ($P = 0.134$). Further analysis indicated two vaccinated animals showed no pyrexia at all (Table 2). Both groups experienced an increase in mean nasal discharge score (Fig. 1C), however, the onset was delayed in the vaccinated group with only one elevated score on day 8 compared with the controls that peaked on days 5 and 7. In the controls, the mean score was significantly higher on day 5 ($P = 0.040$).

4. Discussion

NS3 was administered as a DNA vaccine due to the advantage of expression of native protein in vivo. DNA vaccination has previously been successful in various cattle studies including Nobiron et al. (2003). Partial protection against BVDV was demonstrated after vaccination with a DNA plasmid encoding the glycoprotein E2, and we wanted to investigate whether immunity to non-structural proteins could contribute to protection against viral challenge.

In this study, we observed significant differences between the control and vaccinated groups following virus challenge. Two vaccinated calves demonstrated complete protection, shedding no virus from the nasal mucosa throughout the study. The remaining vaccinates showed greatly reduced virus shedding compared to the controls, where 4/5 shed virus for 4 or more days. The control animals also showed greater increases in rectal temperature and more severe nasal secretions. In terms of clinical efficacy, the NS3 DNA induced levels of protection very similar to vaccination with E2 DNA constructs (Nobiron et al., 2003).

The mechanisms of protection following NS3 vaccination are unclear. Only one animal (calf #13) showed a humoral response after NS3 vaccination, which increased rapidly following challenge indicating a memory response. The Ig isotype of the NS3 specific antibodies induced by vaccination was determined to be IgG1 indicating a bias to a Th2 type immune response as was expected after intradermal delivery of DNA (van Drunen

Little-van den Hurk et al., 1998). The NS3 antibodies were not neutralising. As overall virus clearance from the nasal mucosa was quicker in the NS3-vaccinated group, this suggests NS3 vaccination has enhanced cellular immune responses to the virus. It has been demonstrated that CD4+ T-cells play a role in virus clearance (Howard et al., 1992) in conjunction with neutralising antibodies (Howard et al., 1989). Furthermore, recombinant NS3 protein was significantly recognized by lymphocytes from calves 20 days post-challenge in a study by Lambot et al. (1997) and T-cell epitopes have also been identified in the NS3 protein of the closely related pestivirus Classical Swine Fever Virus (Armengol et al., 2002). A potential T-cell response may also explain the reduced clinical symptoms seen in vaccinated animals that did not seroconvert prior to challenge.

In conclusion, DNA vaccination with NS3 led to comparable protection in all vaccinated animals in terms of clinical symptoms and virus clearance even though only one seroconverted after vaccination. It was evident that anti-NS3 antibodies were not neutralising but virus shedding was still reduced in all vaccinated animals. NS3 does stimulate the immune response and, as it is highly conserved, perhaps has the potential for use in a subunit vaccine to confer humoral and cellular immunity against both type I and II BVDV.

References

Armengol, E., Wiesmuller, K.H., Wienhold, D., Buttner, M., Pfaff, E., Jung, G., Saalmuller, A., 2002. Identification of T-cell epitopes in the structural and non-structural proteins of classical swine fever virus. J. Gen. Virol. 83, 551–560.

Bruschke, C.J., Moormann, R.J., van Oirschot, J.T., van Rijn, P.A., 1997. A subunit vaccine based on glycoprotein E2 of bovine virus diarrhea virus induces fetal protection in sheep against homologous challenge. Vaccine 15, 1940–1945.

Clarke, M.C., Brownlie, J., Howard, C.J., 1987. Isolation of cytopathic and non-cytopathic bovine viral diarrhoea virus from tissues of infected animals. In: Harkness, J.W. (Ed.), Pestivirus Infections of Ruminants. CEC, Luxemburg, pp. 3–10.

Deng, R., Brock, K.V., 1992. Molecular cloning and nucleotide sequence of a pestivirus genome, noncytopathic bovine viral diarrhea virus strain SD-1. Virology 191, 867–879.

Donis, R.O., Corapi, W., Dubovi, E.J., 1988. Neutralising monoclonal antibodies to bovine viral diarrhoea virus bind to the 56 K to 58 K glycoprotein. J. Gen. Virol. 69, 77–86.

van Drunen Little-van den Hurk, S., Braun, R.P., Lewis, P.J., Karvonen, B.C., Baca-Estrada, M.E., Snider, M., McCartney, D., Watts, T., Babiuk, L.A., 1998. Intradermal immunisation with a bovine herpesvirus-1 DNA vaccine induces protective immunity in cattle. J. Gen. Virol. 79, 831–839.

van Drunen Little-van den Hurk, S., Loehr, B.I., Babiuk, L.A., 2001. Immunisation of livestock with DNA vaccines: current studies and future prospects. Vaccine 19, 2474–2479.

Harpin, S., Hurley, D.J., Mbikay, M., Talbot, B., Elazhary, Y., 1999. Vaccination of cattle with a DNA plasmid encoding the bovine viral diarrhoea virus major glycoprotein E2. J. Gen. Virol. 80, 3137–3144.

Howard, C.J., Clarke, M.C., Brownlie, J., 1989. Protection against respiratory infection with bovine virus diarrhoea virus by passively acquired antibody. Vet. Microbiol. 19, 195–203.

Howard, C.J., Clarke, M.C., Sopp, P., Brownlie, J., 1992. Immunity to bovine virus diarrhoea virus in calves: the role of different T-cell subpopulations analysed by specific depletion in vivo with monoclonal antibodies. Vet. Immunol. Immunopathol. 32, 303–314.

Lambot, M., Douart, A., Joris, E., Letessons, J.J., Pastoret, P.P., 1997. Characterisation of the immune response of cattle against non-cytopathic and cytopathic biotypes of bovine viral diarrhoea virus. J. Gen. Virol. 78, 1041–1047.

Nobiron, I., Thompson, I., Brownlie, J., Collins, M.E., 2003. DNA vaccination against bovine viral diarrhoea virus induces humoral and cellular responses in cattle with evidence for protection against viral challenge. Vaccine 21, 2082–2092.