A novel subunit ISCOM vaccine against bovine virus diarrhoea virus

S. KAMSTRUP, L. RØNSHOLT, M.H. JENSEN and K. DALSGAARD *

Summary: The preparation and preliminary testing of a subunit ISCOM (immunostimulating complex) vaccine against bovine virus diarrhoea virus (BVDV) is described. Vaccination of calves with this vaccine yields high neutralising titres against a panel of Danish BVDV field isolates. The serological difference between virus isolates and vaccine strain selection is discussed.

KEYWORDS: Bovine diarrhoea virus - ISCOM - Pestivirus - Vaccines.

Bovine virus diarrhoea (BVD) is an economically important disease of cattle worldwide. In recent years, insight has been gained into the pathogenesis of this disease, and the importance of persistently infected carrier animals has been proven (2, 9). These carrier animals are found in frequencies as high as 1% in dairy and beef herds in Denmark (7). These animals act as sources of infection for pregnant seronegative animals and their foetuses, thereby giving rise to abortions and malformed or persistently infected calves. These persistently infected animals will eventually develop the mucosal disease syndrome. Detection of carrier animals is not easy, and is possible only by demonstrating persistent viraemia.

It is now accepted that immunisation of the dam in order to prevent the foetus from becoming infected would be the most effective way of avoiding the losses caused by this virus. This immunisation may be achieved by vaccinating the dam prior to mating. For this purpose a new subunit vaccine has been developed, based on the ISCOM (immunostimulating complex) technology.

BVD virus (BVDV) grows to only moderate titres in cell culture, and the mass of soluble antigen is generally low compared to other viruses. It has been shown that treatment of virus-infected cells with Triton X-100 releases a large amount of immunologically important antigens, as measured by crossed immunoelectrophoresis (4) or by enzyme-linked immunosorbent assay (ELISA) (L. Rønsholt, unpublished findings). This amount is clearly larger than that released from the cells in the form of viral particles (3). Therefore, the approach adopted by the authors has been to produce large amounts of antigen in cells, release the cell-associated antigen with Triton X-100 and use such antigen for preparing a vaccine. Earlier experiments have shown that this antigen, when properly adjuvanted, gives at least partial protection against infection with BVDV in a challenge experiment (8). To improve these results, the antigens have now been concentrated and partly purified.

* Ministry of Agriculture, State Veterinary Institute for Virus Research, Lindholm, DK 4771 Kalvehave, Denmark.
The adjuvant system used has also been improved. When formulating inactivated vaccines, it is very important to choose a potent adjuvant system. For this purpose Quil A has been chosen, as this saponin has proved to be one of the strongest adjuvants for presenting membrane associated antigens to the immune system (1).

The antigen extracted using Triton X-100 is chromatographed on a new type of chromatographic medium called “Matrex Cellufine Sulphate” (Amicon). This material preferentially binds the immunogenic proteins of BVDV proteins rendered soluble by Triton X 100 treatment and complies with good manufacturing practice. The matrix is autoclavable, withstands 0.1 M sodium hydroxide and formaldehyde, and does not bind pyrogens, bovine serum albumin or bovine immunoglobulins, which means that these contaminants can be washed away easily. The process is easy to scale up.

Once the antigens are bound to the column and washed, they may be eluted by a single buffer step. By exchanging the Triton X-100 buffer with a buffer containing Quil A, an elution step has been successfully developed during which ISCOMs are formed. An ISCOM consists of antigens inserted into a matrix formed by Quil A, cholesterol and phosphatidylcholine (6). The technique used is a simple industrial process for the production of ISCOMs. It has major technical advantages over the more sophisticated techniques commonly applied in laboratory-scale productions. To date, there is only one commercially available ISCOM vaccine (against equine influenza virus).

The authors have prepared ISCOM vaccines against BVDV, using two different Danish strains of BVDV for antigen production. The two strains (Ug59 and 258) are both of the cytopathogenic biotype. They were selected because they represent quite different variants of the antigenic spectrum of BVDV. Traditionally, no distinct serotypes have been defined for BVDV, although individual isolates may show considerable differences in cross neutralisation tests.

In testing these vaccines, three groups of three animals each were vaccinated twice, three weeks apart, with vaccine based on either monovalent antigen (Ug59 or 258) or bivalent antigen (Ug59 and 258 vaccines in combination). Serum samples taken two weeks after the second injection were tested in a virus neutralisation assay against a number of Danish BVDV isolates (Fig. 1).

Considerable differences in neutralisation of individual Danish field isolates of BVDV were observed, with serum neutralising titre (SNT) values for the Ug59-based vaccine differing by up to 30 times against the serologically most distant isolates (Ug59 and 10039). However, the neutralising titres are generally much higher than previously obtained with inactivated vaccines, and the vaccines used in this study were active against all isolates tested. The animals vaccinated with both strains of BVDV (bivalent vaccine) show SNT values as high or higher than the animals in the other two groups. Comparing the two monovalent vaccines, it seems that the vaccine based on strain 258 induces a slightly better overall reaction across the range of BVDV isolates used in the test.

Further experiments are needed to determine whether a monovalent vaccine is sufficiently effective.

The ultimate test for this vaccine is a challenge experiment in pregnant cattle, to determine whether the vaccine can protect against intrauterine infection. However, there is circumstantial evidence that significant levels of neutralising antibodies in the dam are capable of protecting the foetus (5, 8). Since BVDV produces a viraemic
Serum neutralising titres (SNT) *

Virus isolate neutralised
- vaccine based on Ug59
- vaccine based on Ug59 and 258
- vaccine based on 258

* SNT obtained with immunostimulating complex (ISCOM) vaccines, measured against a number of Danish field isolates (titres given as the reciprocal of the highest dilution neutralising 100 TCID$_{50}$)

FIG. 1

Vaccination of cattle against bovine virus diarrhoea virus with a subunit Quil A/ISCOM vaccine

stage early after infection, such a correlation would seem reasonable. At present, no minimum protective SNT value has been ascertained, although it has been suggested that there might be a certain critical level below which there is no protection (8).

In conclusion, a subunit ISCOM vaccine against BVDV has been developed, capable of inducing high serum neutralising titres not only against homologous strains but also against a number of different Danish field isolates. The preparative procedure is suitable for scaling up and is based on only a few steps. The vaccine is currently being tested in clinical trials.
série de souches du VDVB trouvées au Danemark. Dans la discussion, les auteurs abordent les différences sérologiques entre les souches virales isolées sur le terrain et les souches vaccinales sélectionnées.

MOTS CLÉS : ISCOM - Pestivirus - Vaccins - Virus de la diarrhée virale bovine.

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UNA NUEVA VACUNA COMPLEJA INMUNOESTIMULANTE (ISCOM) SUBUNITARIA CONTRA EL VIRUS DE LA DIARREA VÍRICA BOVINA. S. Hamstrup, L. Ronsholt, M.H. Jensen y K. Dalsgaard.

Resumen: Los autores describen la preparación y las pruebas preliminares de una vacuna compleja inmunoeestimulante (ISCOM) subunitaria contra el virus de la diarrea vírica bovina. La vacunación de terneros con esta vacuna produce elevados títulos neutralizantes contra un grupo de virus de la diarrea vírica bovina daneses aislados en el campo. El artículo también comenta la diferencia serológica entre los virus aislados y una selección de cepas vacunales.

PALABRAS CLAVE: ISCOM - Pestivirus - Vacunas - Virus de la diarrea vírica bovina.

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REFERENCES


