



Maternal antibody blocks humoral but not T cell responses to BVDV

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Abstract

Bovine viral diarrhoea virus (BVDV) contributes significantly to health-related economic losses in the beef and dairy industry. Antibodies of maternal origin can be protective against BVDV infection, however, calves with low titres of maternal antibody or that do not receive colostrum may be at risk for acute BVDV infection. Interference by high titres of maternal antibodies prevents the development of an antibody response following vaccination with either a killed or attenuated BVDV vaccine. However, the T cell mediated immune response to BVDV may be generated in the absence of a detectable serum neutralizing antibody response. Two trials were conducted to evaluate the potential to elicit T cell mediated immune responses to BVDV in calves with circulating maternal antibody to BVDV. In the first trial, calves with high levels of circulating maternal antibody to BVDV 1 and BVDV 2 were experimentally infected with BVDV 2 (strain 1373) at two to five weeks of age. The T-cell mediated immune responses of the experimentally infected calves and non-infected calves were monitored monthly until circulating maternal antibody was no longer detectable in either treatment group. Calves experimentally infected with BVDV developed BVDV specific CD4⁺, CD8⁺, and δ T cell responses while high levels of maternal antibody were circulating. A second challenge with BVDV 2 (strain 1373) was performed in the experimentally infected and control calves once maternal antibody could no longer be detected. Previous exposure to BVDV in the presence of maternal antibody protected calves from clinical signs of acute BVDV infection compared to the control calves. In the second trial, three groups of calves with circulating maternal antibody to BVDV were given either a modified live vaccine (MLV) containing BVDV 1 and BVDV 2, a killed vaccine containing BVDV 1 and BVDV 2, or no vaccine, at seven weeks of age. Serum neutralizing antibody levels and antigen specific T cell responses were monitored for 14 weeks following vaccination. Calves vaccinated with MLV BVDV developed BVDV 1 and BVDV 2 specific CD4⁺ T cell responses, and BVDV 2 specific $\gamma\delta$ T cell responses, in the presence of maternal antibody. Vaccination with killed BVDV did not result in the generation of measurable antigen specific T cell immune responses. In this trial, a second vaccination was performed at 14 weeks to determine whether an anamnestic antibody response could be generated when calves were vaccinated in the presence of maternal antibody. Calves vaccinated with either a MLV or killed BVDV vaccine while they had maternal antibody developed an anamnestic antibody response to BVDV 2 upon subsequent vaccination. The results of these trials indicate that vaccinating young calves against BVD while maternal antibody is present may generate BVDV specific memory T and B cells. The data also demonstrated that seronegative calves with memory T and B cells specific for BVDV may be immune to challenge with virulent BVDV.

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Keywords: BVDV; T lymphocyte subsets; Maternal antibody; Vaccination

1. Introduction

Bovine viral diarrhoea virus (BVDV) contributes significantly to health-related economic losses in the beef

and dairy industries. Maternal antibody derived from colostrum, if present in high enough titre, can protect calves from clinical signs of BVDV infection. However, the maternal antibody also prevents calves from producing an antibody response to infection or vaccination with modified live or killed vaccines [1]. Calves are born agammaglobulinaemic and depend heavily on ingestion of colostrum antibodies for survival [2]. In addition, the specific and non-specific immune systems are immature

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in newborn calves, making them especially vulnerable to infections during the first weeks of life. Stimulating a protective immune response in the calf while it still has a protective level of maternal antibody would be very beneficial. The calves would transition from maternally derived immunity to long lasting acquired immunity without experiencing a period of vulnerability before a vaccine can induce protection.

Circumvention of vaccine interference by maternal antibody is currently being explored through the use of new adjuvant technology, epitope modification, and non-conventional routes of delivery [3]. Conventional vaccines, however, may also be able to stimulate immune memory in the face of maternal antibody. The development of an immune response to vaccination is usually measured by the presence or absence of antibodies in the blood of the vaccinated animal. Assays that measure antibodies detect humoral immunity, but give no information about T cell immunity. In general, T helper cell (CD4⁺ T cell) responses are assumed to occur concurrently with humoral responses due to the requirement for T cell help in generation of a humoral response. Cytotoxic T cells (CD8⁺ T cells), gamma delta ($\gamma\delta$) T cells, and memory B cells may also play very important roles in immunity. Measuring serum antibody does not provide a complete picture of the animal's immune response because the activity of these important cells is not evaluated.

Antigen-specific up-regulation of the CD25 molecule on the surface of T cells has proven to be a useful marker of antigen specific T cell activation in cattle [4–6]. The CD25 molecule is the high affinity alpha subunit of the interleukin 2 (IL2) receptor. Lymphocytes which have recognized their specific antigen will up-regulate their surface expression of CD25. This allows binding of IL2 to the IL2 receptor thereby inducing lymphocyte mitosis and expansion of antigen specific clones of lymphocytes. A flow cytometer can be used to evaluate up regulation of CD25 on T cell subsets after exposure to antigen. This allows the determination of antigen specific CD4⁺, CD8⁺, and $\gamma\delta$ T cell responses to vaccination.

2. BVDV specific T cell responses in calves with maternal antibody

Two trials were conducted to evaluate the potential to elicit T cell mediated immune responses to BVDV in calves with circulating maternal antibody. One trial involved experimental challenge with virulent BVDV and the other involved vaccination. The CD25 activation marker was used to detect the development of antigen specific T lymphocytes following in vitro stimulation with BVDV 1 and BVDV 2. In the first trial, calves with high levels of circulating maternal antibody

to BVDV 1 and BVDV 2 were either experimentally infected with BVDV 2 (strain 1373) at two to five weeks of age or not infected. Serum neutralizing antibodies to BVDV from infected and non-infected calves declined steadily following ingestion of colostrum, indicating the lack of a detectable humoral immune response to BVDV challenge. The T cell mediated immune responses of the experimentally infected calves and non-infected calves were monitored monthly until circulating maternal antibody was no longer detectable in either treatment group. Calves experimentally infected with BVDV at two to five weeks of age developed BVDV specific CD4⁺, CD8⁺, and $\gamma\delta$ T cell responses while high levels of maternal antibody were circulating. A second challenge with BVDV 2 (strain 1373) was performed in the experimentally infected and control calves once maternal antibody could no longer be detected. Previous exposure to BVDV in the presence of maternal antibody protected calves from clinical signs of acute BVDV infection compared to the control calves.

In the second trial, three groups of calves with circulating maternal antibody to BVDV were given either a modified live vaccine (MLV) containing BVDV 1 and BVDV 2, a killed vaccine containing BVDV 1 and BVDV 2, or no vaccine, at seven weeks of age. Serum neutralizing antibody levels and antigen specific T cell responses (as evaluated by up regulation of CD25 after in vitro exposure to antigen) were monitored until 14 weeks following vaccination. Vaccination at seven weeks of age with either MLV or killed vaccine did not generate any detectable increase in serum neutralizing antibodies specific for BVDV. Calves vaccinated with MLV BVDV developed significant ($P<0.05$) BVDV 1 and BVDV 2 specific CD4⁺ T cell responses, and BVDV 2 specific $\gamma\delta$ T cell responses, in the presence of maternal antibody. Vaccination with killed BVDV did not result in the generation of significant antigen specific T cell immune responses. In this trial, a second vaccination with MLV was performed at 14 weeks after vaccination to determine whether an anamnestic antibody response could be generated when calves were vaccinated in the presence of maternal antibody. Interestingly, calves vaccinated with either an MLV or killed BVDV vaccine while maternal antibody circulated developed a memory antibody response to BVDV 2 upon subsequent vaccination.

3. Conclusions

The results of these trials indicate that infecting or vaccinating young calves with BVDV while maternal antibody is present is capable of generating memory B cells, and that infection or MLV vaccine are capable of stimulating T cell mediated responses to BVDV. Similar results have been reported for bovine herpesvirus 1

(BHV-1) and bovine respiratory syncytial virus (BRSV) vaccine administered in the face of maternal antibody. Calves vaccinated against BHV-1 are unable to produce a measurable humoral response until maternal antibody has waned to undetectable levels [1]. In this same report, however, calves vaccinated while maternal antibody levels were high demonstrated an anamnestic humoral response to BHV-1 following a later vaccination. Newborn calves vaccinated with BRSV also have an enhanced humoral response to a second vaccination with BRSV, although no humoral response was detected following the first inoculation in the face of maternal antibody [7]. These memory responses indicate that helper T cells were stimulated to induce memory B cells in response to vaccination. T lymphocyte proliferation in response to *in vitro* exposure to BHV-1 and BRSV has been demonstrated in calves vaccinated against these viruses while they still had maternal antibody, even though no humoral immune response was detected [8,9]. Antigen specific T cell proliferation is interpreted as an indication of T cell mediated immunity, although it does not provide information on the type of T cell responding.

The results summarized here indicate that calves exposed to BVDV while they still have maternal antibody can develop memory CD4⁺, CD8⁺, and $\gamma\delta$ T cell responses and memory B cell responses even though they do not produce an antibody response. Virulent virus was more efficient at inducing T cell memory than were vaccines, and the MLV vaccine was more efficient than the killed vaccine. Calves with memory B and T cells can lose their maternal antibody and become seronegative for BVDV antibody. After they become seronegative, the calves can be completely resistant to a BVDV challenge which makes other seronegative calves clinically ill. This means that it is not sufficient to test

calves for the presence of BVDV antibody to determine if they are susceptible to challenge with BVDV. When conducting vaccination challenge experiments, it is essential to test for T cell mediated immunity to BVDV to be sure that the animals are immunologically naïve to BVDV and are susceptible to infection.

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