Immunological responses to bovine virus diarrhoea virus infections

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Summary: Infection of normal calves with bovine virus diarrhoea virus (BVDV) is a transient self-limiting infection that can result in a period of immunosuppression. The virus appears to be able to replicate in all of the major lymphocyte sub-populations as well as in accessory cells. This may result in the leukopenia that is often a sequel of infection and affects B-cells as well as the T-cell sub-populations expressing either BoCD4 or BoCD8 antigens. B and T-cell responses are affected as a consequence of exposure to BVDV and there is a reduced ability to control other infections. Evidence is summarised and shows that immunoglobulin is an important mediator of immunity to infection with BVDV. Although the foetus can mount an immune response in the latter part of gestation, during the first trimester it does not. A specific state of tolerance is induced and this is associated with change in the proportion of certain lymphocyte sub-populations and ability to respond to immune stimulation.


The outcome of infection with bovine virus diarrhoea virus (BVDV) can, in simplest terms, be considered to be under two major influences. One is the immunological maturity of the foetus and the other is the state of active or passive immunity which exists in the individual or herd. Infection with BVDV in normal calves or adult cattle is transient and self-limiting. The virus spreads laterally and the infection has many of the characteristics of other acute virus infections in that it is often mild and subclinical but when it occurs as part of a complex syndrome, involving several micro-organisms, it can contribute to a disease that is clinically evident. An example is the respiratory disease complex seen in both intensively housed calves as well as in older feedlot animals. The mild self-limiting infection results in the generation of an immune response and is quite distinct from the acute onset of disease, seen in immunotolerant animals, that develops into mucosal disease and death within a few weeks. Mucosal disease involves, as its cornerstone, a specific immunological tolerance which results from infection of the immature foetus with BVDV (see the chapter in this issue on pathogenesis).

Although the major route of viral transmission in groups of intensively reared cattle is almost certainly respiratory, the infection that follows is not limited to this
local site. Virus spreads systemically and can be recovered from the blood as well as from secretions and many lymphoid and other tissues (Howard, Clarke and Brownlie, unpublished results).

**Antibody response to acute infection**

Upon either natural or experimental infection with BVDV and subsequent recovery, a serum antibody response is generated (32). This antibody response can be detected by a variety of assays (see the chapter in this issue on diagnostic methods). Several publications have documented ELISA as being a sensitive and rapid method to demonstrate the presence of antibodies or for monitoring their occurrence subsequent to infection (6, 13, 22, 25, 27). These assays have depended on antigen extracted from virus infected cultures, partially purified viral preparations or have used fixed infected cells.

ELISA is also useful for assessing the contribution of the various isotypes to the response. Although it has been suggested that IgM antibodies are the predominant class produced (20), subsequent studies (22) have shown that the majority of serum antibodies are IgG1 and IgG2 and this is the typical response following the majority of other infections of cattle. It has long been known that a neutralising antibody response is generated as a result of infection. With BVDV both cytopathogenic and non-cytopathogenic biotypes can be neutralised and both IgG1 and IgG2 antibodies are effective in the absence of complement.

The time course of the antibody response, to infection with BVDV, is different from that seen in many other acute virus infections of young calves. Thus, although antibody can be detected in serum three weeks after infection, the titre continues to rise for 10 to 12 weeks; after this time a plateau is reached which is followed by a slow decline (24). The relatively slow increase in antibody titre may, in part, be due to an immunosuppressive effect by the virus (see below). The long time taken to reach a plateau is interesting since virus shedding and leukopenia are transient and rarely last longer than two weeks post infection. It is possible that the virus persists in the tissues for longer than this. Both cytopathogenic and non-cytopathogenic biotypes produce a similar antibody response in young gnotobiotic calves whether it is monitored by the neutralisation assay or by ELISA.

Immunoblotting techniques have been applied to the study of which viral antigens are recognised by the host and it appears that all are antigenic (16, 35). The 56-58 kDa surface glycoprotein appears to be the major target for neutralisation by antibody (17, 31) although antibodies to other polypeptides may also be effective (33).

**Immunity to acute infection**

It is generally accepted that, following infection, a serum antibody response occurs and that animals are immune to re-infection (37). However, where individual animals have been monitored it has been noted that after a few months the titre in serum declines and animals can subsequently show an increase in antibody (11). This is presumably due to another infection and indicates that immunity following natural infection is not lifelong. The implication arising from this observation is that immunity will also be of limited duration following vaccination with live virus, which can be regarded as simply an artificial infection.
The classical way to demonstrate that antibody mediates immunity to infection is by passive transfer of serum, or immunoglobulin purified from it, and determination of the effect on viral replication or disease. House and Manley (21) noted that passive antibody given subcutaneously alleviated the leukopenia and temperature response that resulted from intranasal challenge with BVDV. In other experiments Shope et al. (40) observed that passively acquired colostral antibody prevented a fatal viraemia in calves immunosuppressed with dexamethasone. Further experiments (23) demonstrated that passively acquired, BVDV specific, colostral antibody protected against infection following intranasal challenge. This was indicated by reduced nasal shedding of virus, a reduction in viraemia and a decreased number of calves with leukopenia. However, although infection was clearly modulated, animals were not totally refractory to infection as an IgG2 antibody response to BVDV could be demonstrated in calves that had been the recipients of the passive IgG1 antibody. Taken together these observations leave no doubt that immunoglobulin is an important mediator of immunity to infection or re-infection with BVDV.

The direct role of cell-mediated responses is more difficult to assess, particularly in outbred cattle where adoptive transfer of cells is not possible. To overcome this problem one approach has been the investigation of the potential role of cytotoxic T-cells, in recovery from infection with BVDV, by specific depletion of lymphocyte sub-populations. This was achieved in vivo by inoculation of calves with murine monoclonal antibodies directed against the bovine CD4 or CD8 antigens and determination of the effect of this treatment on infection (23). Depletion of BoCD8+ lymphocytes had no effect on infection but following depletion of BoCD4+ lymphocytes viraemia was prolonged. Thus, no evidence was obtained to indicate that MHC class I restricted cytotoxic T-cells play a pivotal role in resolution of infection.

**Immunosuppressive properties of BVDV**

Several field studies have reported that where BVDV is associated with other microorganisms clinical disease in cattle appears more severe. Experimental data have confirmed that prior infection with BVDV compromises the animals and allows a second infectious agent to replicate more extensively and in some cases produce more severe disease (18, 36, 38, 45). It has been suggested that this increased susceptibility to secondary infection is because of an immunosuppressive effect by the virus.

Following infection a transient leukopenia is usually evident. Studies by Bolin et al. (7) showed that after intravenous challenge with a cytopathogenic strain of BVDV the number of both T and B-cells in peripheral blood was decreased but the T-cells were most severely affected. Studies in vitro have shown that BVDV can replicate in lymphocytes and macrophages and affect certain of their responses (28, 42). Furthermore, immunohistochemical staining has shown the viral antigen to be present primarily in the lymphoid tissues of experimentally infected, immunologically mature foetuses and, interestingly, in the antigen presenting cells (2). Further studies reported by Ellis et al. (19) demonstrated that intranasal infection with a non-cytopathogenic strain of BVDV resulted in a transient leukopenia. A decrease in the absolute numbers of circulating T-lymphocytes, including BoCD4+ and BoCD8+ subsets, as well as B-cells and neutrophils was noted. No significant variation in the numbers of monocytes or of T-cells, expressing the BoWC1 215/300 kDa antigen, was seen. Thus, it would appear that the virus is somewhat catholic in its taste for cells and may well affect a variety of lymphocytes and accessory cells.
Several investigators have monitored immune functions either in vivo or in vitro after infection with BVDV. Lamontagne et al. (29) reported that lambs infected with a cytopathogenic strain of BVDV showed a modulated response, to lectins, that varied with time after infection. A depressed response to T-lymphocyte stimulation was noted for peripheral blood mononuclear cells taken from cattle exposed to BVDV (38). Infection has also been shown to affect responses to mitogens (34) and the in vitro synthesis of immunoglobulins (1). Taken together these reports support the view that acute infection with BVDV has a role in microbiologically complex diseases because of its immunosuppressive properties that predispose the animal to other infectious microbes.

Immune response following foetal infection

Experimental or natural infection of the foetus, with a non-cytopathogenic biotype of BVDV before about day 110 of gestation and prior to the development of immunological competence, results in virus growth in the foetal tissue. No specific immune response is mounted and the calves are born persistently infected (a lifelong state) and tolerant to BVDV (9, 26). In the least complicated scenario, calves are born apparently healthy but with this tolerance which is specific to the persisting virus. The calves can respond normally to other antigens and infectious microbes and to other strains of BVDV (41, 44) and this is presumably a response to unshared epitopes. It is apparent that foetal infection can damage the developing lymphoid tissue and there are many reports of impaired immune responses in viraemic calves or an increased susceptibility to other infections (34, 39). Thus, although the specific tolerance is necessary for development of mucosal disease (see the chapter on pathogenesis) the potential problems that can occur as a result of foetal infection are much more complex. It has been observed that inoculation of the early foetus with a cytopathogenic strain of virus did not result in virus persistence and no immune response was generated (10).

The question arises as to whether specific cell populations are differently affected in viraemic cattle. Immunohistological and other studies (3, 4, 5, 8) have shown that viral antigen was associated with all the major lymphocyte sub-populations, various accessory cell types and cells that do not have immune functions.

In a comparison of normal sheep and animals that were persistently infected, with Border disease virus, Burrells et al. (12) reported a significant increase in the proportion and absolute numbers of B-cells that expressed surface Ig, MHC class II antigens or ovine CD1. The proportion of T-cells in the circulation was reduced but not the absolute numbers. Muscoplat et al. (34) reported decreased numbers of B-cells in two cattle with chronic clinical BVDV while Larsson et al. (30) noted an increased number in animals with clinical mucosal disease. A comparison of calves, infected in utero with a non-cytopathogenic strain of BVDV (Pe515nc), and age matched controls (Howard, Brownlie and Clarke, unpublished results) indicated that there was evidence of a leukopenia in a number of these animals compared to controls. The proportion of B-cells that reacted with monoclonal antibody to the BoWC3 antigen or to IgM was normal (Table I). The percentage of T-cells expressing the bovine CD2, CD4, CD6 and CD8 antigens was not different from control values but a significant decrease in the ratio of T-cells expressing the 215/300 kDa BoWC1 antigen was noted. This contrasts with findings in acutely infected cattle noted above. The biological relevance of this observation is not known but these cells express the
### TABLE I

**Comparison of lymphocyte sub-populations in normal and viraemic cattle**

<table>
<thead>
<tr>
<th>Cells stained with monoclonal antibody to</th>
<th>Percentage of peripheral blood mononuclear cells identified in calves</th>
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<tbody>
<tr>
<td></td>
<td>Normal (n = 6) (^1)</td>
</tr>
<tr>
<td>BoCD2 (^2)</td>
<td>39 (33-45) (^3)</td>
</tr>
<tr>
<td>BoCD6</td>
<td>35 (30-41)</td>
</tr>
<tr>
<td>BoCD4</td>
<td>20 (16-25)</td>
</tr>
<tr>
<td>BoCD8</td>
<td>11 (7-19)</td>
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<tr>
<td>BoCD5</td>
<td>64 (59-71)</td>
</tr>
<tr>
<td>BoWC1</td>
<td>27 (21-40)</td>
</tr>
<tr>
<td>BoWC3</td>
<td>18 (14-23)</td>
</tr>
<tr>
<td>Ig</td>
<td>23 (16-27)</td>
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</tbody>
</table>

1. Six normal and five viraemic calves compared.
2. Monoclonal antibodies to indicated bovine CD antigens (BoCD) used. BoWC1 = MAb CC15 to p 215/300 kDa antigen on BoCD4-, BoCD8-, T-cells. BoWC3 = MAb CC21 to p 145 kDa antigen on B-cells.
3. Mean and range shown; only BoWC1 showed a significant difference, p<0.05.

Consideration should also be given to the possible role of the immune response in the damaging effects that result from infection of the foetus (15, 43). When this occurs at the same time that immunocompetence is developing, it might result in an imbalanced immune response that plays a role in causing some of the severe defects observed in foetuses that have been exposed to BVDV. As noted above, the foetus can mount an active immune response if infected after immunocompetence has developed.

In conclusion, foetal infection may result in the birth of calves that are specifically immunotolerant and viraemic but otherwise apparently healthy.

It may also result in more extensive damage to the immune system and has the potential to reduce an animal’s ability to respond to infectious or other antigens.

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**RÉPONSES IMMUNOLOGIQUES AUX INFECTIONS PAR LE VIRUS DE LA DIARRHÈE VIRALE BOVINE.** – C.J. Howard.

**Résumé:** Le virus de la diarrhée virale bovine (BVDV) provoque, chez le veau normal, une infection passagère et autolimitante qui peut entraîner une phase d'immunodépression. Le virus apparaît capable de se réplicer aussi bien dans...
toutes les sous-populations de lymphocytes principalement responsables de la réponse immunitaire, que dans les cellules ayant un rôle accessoire. La leucopénie qui peut en résulter est souvent une séquelle de l'infection ; elle intéresse aussi bien les sous-populations de cellules B que celles de cellules T qui expriment soit l'antigène BoCD4 soit l'antigène BoCD8. Les réponses des cellules B et T sont diminuées par suite de l'exposition au virus de la BVD et leur capacité de contrôle d'autres infections s'en trouve réduite. Il est démontré que l'immunoglobuline a un rôle important de médiateur de l'immunité vis-à-vis de l'infection par le BVDV. La réponse immunitaire du fœtus, possible dans la dernière phase, ne l'est pas pendant le premier trimestre de la gestation. La tolérance spécifique induite est liée au changement intervenu dans la proportion de certaines sous-populations de lymphocytes et dans la capacité de réponse à la stimulation immunitaire.


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RESPUESTAS INMUNOLÓGICAS A LAS INFECCIONES POR VIRUS DE LA DIARREA VIRAL BOVINA. – C.J. Howard.

Resumen: El virus de la diarrea viral bovina provoca en terneros normales una infección autolimitada y transitoria que puede dar lugar a una fase de inmunodepresión. El virus parece capaz de duplicarse en todas las subpoblaciones más importantes de linfocitos y en células accesorias. Esto puede entrañar una leucopenia, consecuencia frecuente de la infección que afecta a células B así como a subpoblaciones de células T que manifiestan antígenos BoCD4 ó BoCD8. Las respuestas de células B y T son afectadas como consecuencia de la exposición al virus y su capacidad de control de otras infecciones es reducida. Existen datos suficientes que demuestran que la inmunoglobulina es un mediador importante de la inmunidad frente a la infección por el virus. Si bien el feto es capaz de respuesta inmune en la última fase de la gestación, no lo es durante el primer trimestre. La tolerancia específica inducida es asociada con cambios en la proporción de ciertas subpoblaciones de linfocitos y capacidad de responder a la estimulación inmunitaria.

PALABRAS CLAVE: Anticuerpo - Anticuerpos monoclonales - Infección aguda - Infección fetal - Inmunodepresión - Respuesta de linfocitos - Respuesta inmunitaria - Subconjunto de linfocitos - Tolerancia - Virus de la diarrea viral bovina.

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REFERENCES


