Antigen-presenting cells from calves persistently infected with bovine viral diarrhoea virus, a member of the Flaviviridae, are not compromised in their ability to present viral antigen

E. Jane Glew and Chris J. Howard

Institute for Animal Health, Compton, Newbury, Berkshire RG20 7NN, UK

The aim of this study was to assess whether the infection of antigen-presenting cells (APC) in vivo, evident in calves persistently infected (PI) with bovine viral diarrhoea virus (BVDV), compromised their ability to stimulate virus-specific T cell responses. Major histocompatibility complex (MHC) molecule-identical cattle were identified from the inbred family at the Institute for Animal Health. One was PI and immunotolerant to BVDV. Virus was not isolated from the remaining calves, which were classified as BVDV-immune or BVDV-naïve depending on the presence or absence of BVDV-specific antibodies in sera. Two-colour flow-cytometric analysis of PBMC from the PI calf showed that 40% of CD14<sup>+</sup> monocytes were infected in vivo. Monocytes from the PI calf (PI monocytes) were used as naturally infected ex vivo APC with CD4<sup>+</sup> or CD8<sup>+</sup> T cells isolated from the BVDV-naïve or BVDV-immune animals. PI monocytes stimulated proliferative responses with CD4<sup>+</sup> and CD8<sup>+</sup> T cells from BVDV-immune animals, but not from BVDV-naïve calves. This provided evidence for the presence of virus-specific CD4<sup>+</sup> and CD8<sup>+</sup> memory T cells after acute infection and indicated that ex vivo monocytes from PI, immunotolerant calves stimulated both MHC class I- and MHC class II-restricted T cell responses to BVDV. Additionally, naturally infected ex vivo monocytes cultured in vitro for 3 days stimulated effective T cell responses to the virus with which they were infected.

Introduction

Bovine viral diarrhoea virus (BVDV) is a single-stranded RNA virus, classified within the family Flaviviridae. It infects a large proportion of cattle worldwide and causes a number of clinical diseases ranging from subclinical infection to acute fatal mucosal disease (MD) (Baker, 1995; Houe, 1995). Both non-cytopathic (ncp) and cytopathic (cp) biotypes are evident, based upon the effects of virus on cells in tissue culture (Meyers & Thiel, 1996).

Infection of animals with ncp BVDV generally causes a transient infection, with the animal exhibiting subclinical or mild symptoms of infection (Baker, 1995). However, if an animal becomes infected with ncp virus within the first trimester of pregnancy, virus can cross the placenta and infect the foetus (Brownlie et al., 1984; Fredriksen et al., 1999; Moennig & Liess, 1995). This may result in the birth of a calf that is persistently infected (PI) and specifically immunotolerant to the infecting virus. Very little or no antibody to BVDV is evident in sera, and viral antigen has been reported to be widely distributed in tissues, most notably in cells and organs of the immune system (Bielefeldt Ohmann, 1988; Bielefeldt Ohmann et al., 1987; Sopp et al., 1994). Superinfection of PI calves with a cp homologous strain of BVDV can cause death due to the onset of MD (Brownlie et al., 1984). Calves PI with BVDV may suffer from reduced weight gain, growth retardation and higher rates of neonatal mortality, usually through secondary infection with enteric or respiratory pathogens. Additionally, PI animals are reported to suffer from a degree of immunosuppression, although the molecular basis for this has not yet been defined (Johnson & Muscoplat, 1973; Muscoplat et al., 1973; Polgieter, 1995).

Many viruses are reported to subvert the normal host immune response during infection. This can aid the establishment and maintenance of viable infection within the host and limit the level of immune-mediated damage to host tissues. Several mechanisms by which viruses alter the immune response of the host have been described. These include virus latency, infection of immunoprivileged sites, synthesis of cytokine homologues and receptors, mutation of the viral genome, which subsequently prevents binding of viral peptides to host MHC class I and II molecules, inhibition of antigen processing and presentation pathways and interference with...
the host cellular machinery (reviewed by Spriggs, 1996; Tortorella et al., 2000). A number of these effects have direct consequences on the ability of the antigen-presenting cells (APC) to stimulate an immune response.

BVDV has been reported to modulate functions of immune cells after infection in vitro, with increased production of nitric oxide from infected macrophages (Adler et al., 1994), decreased production of TNFα from lipopolysaccharide (LPS)-stimulated macrophages (Adler et al., 1996) and reduction of Fc and C3 receptor expression on, and phagocytic activity of, alveolar macrophages (Welsh et al., 1995). Additionally, several authors have reported that cells, isolated from PI animals, that are pivotal in control of the immune response are infected in vivo. These include the antigen-presenting myeloid cells, CD4+ and CD8+ T lymphocytes and B cells (Bielefeldt Ohmann et al., 1987; Bielefeldt Ohmann, 1988; Bruschke et al., 1998; Sopp et al., 1994). However, it has not been established whether APC from PI, specifically immunotolerant, cattle are compromised in their ability to induce immune responses to the virus. Any effect might play a role in the pathogenesis of MD or in the generalized immunosuppression noted in PI cattle.

A breeding programme at the Institute for Animal Health has produced cattle that are major histocompatibility complex (MHC) identical. A series of experiments was performed using T cells from cattle that were immune to BVDV, or that had not come into contact with the virus, and APC from an MHC-identical, PI animal, to determine whether being persistently infected compromised the ability of APC to present BVDV antigen to T cells.

**Methods**

- **Experimental animals.** Calves (*Bos taurus*) were conventionally reared British Holstein Friesians bred at the Institute for Animal Health. All were from the same family of animals and were MHC identical, having the same haplotype (A18~A31; Ellis et al., 1998). The immune status of each animal was determined by assaying sera by ELISA for antibody to BVDV (Howard et al., 1985) and by testing for persistent viraemia by indirect immunofluorescence (Brownlie et al., 1984). Cattle were classified as being in one of three groups. Calves that were not PI with BVDV and that had no serum antibody to BVDV were considered not to have been exposed to virus and were considered BVDV-naïve (n = 3). Cattle that were not PI with virus but that had serum antibody to BVDV were considered to have been exposed to the virus and to have recovered from infection (n = 4). These were termed BVDV-immune and had been housed previously with the PI calf (n = 3) or had acquired infection naturally from another source and seroconverted (n = 1). One animal was identified that was PI and had no serum antibody. Subsequent to their identification, all three groups of cattle were housed in separate secure accommodation that maintained the immune status of the animals, which was checked periodically throughout the experimental period. The procedures were approved by the Institute’s ethics committee.

- **Cell separation, culture and storage.** PBMC were separated by density gradient centrifugation (1-083 g/ml Histopaque; Sigma) from blood taken into heparin (10 units per ml blood; Leo). Cells were resuspended in tissue culture medium (TCM) consisting of RPMI-1640 medium with glutamax I (Life Technologies), supplemented with 10% heat-inactivated FCS, 5 × 10⁻³ M 2-mercaptoethanol and 50 µg/ml gentamycin. Monocytes were isolated from PBMC, taken from the PI calf, after staining with an anti-CD14 monoclonal antibody (MAb), CC-G33 (Table 1), and incubation with anti-mouse IgG1 super-paramagnetic particles (Miltenyi Biotec). Labelled cells were isolated using a MiniMacs column (Miltenyi Biotec). Following the manufacturer’s instructions. The purity of the cells was evaluated by flow cytometry and shown to be > 96%. CD4+ and CD8+ T lymphocytes were obtained in a similar manner from non-PI calves that were seropositive (immune) or seronegative (naïve) using anti-CD4 or anti-CD8 MAbs, respectively (Table 1). For convenience, these T cell populations are referred to as ‘immune’ or ‘naïve’.

Monocytes (CD14+), obtained by paramagnetic isolation, were either resuspended at 10⁵ per ml in TCM and used directly in proliferation

**Table 1. Specificity of MAbs**

MAbs used for immunofluorescent staining and for isolating monocytes (CD14+), CD4+ and CD8+ T cells by paramagnetic sorting are listed. All antibodies had previously been titrated on PBMC to give optimal staining by FACS.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Isotype</th>
<th>Specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC-G33</td>
<td>IgG1</td>
<td>Bovine CD14</td>
<td>Sopp et al. (1996)</td>
</tr>
<tr>
<td>CC65</td>
<td>IgG2a</td>
<td>Bovine CD8</td>
<td>Sopp et al. (1994)</td>
</tr>
<tr>
<td>CC55</td>
<td>IgG1</td>
<td>Bovine CD8</td>
<td>MacHugh et al. (1991)</td>
</tr>
<tr>
<td>CC8</td>
<td>IgG2a</td>
<td>Bovine CD4</td>
<td>Sopp et al. (1994)</td>
</tr>
<tr>
<td>CC15</td>
<td>IgG2a</td>
<td>WC1*</td>
<td>Sopp et al. (1994)</td>
</tr>
<tr>
<td>IL-A58</td>
<td>IgG2a</td>
<td>IgG light chain</td>
<td>Davis et al. (1996)</td>
</tr>
<tr>
<td>GB21A</td>
<td>IgG2b</td>
<td>Bovine γδTCR</td>
<td>Sopp et al. (1994)</td>
</tr>
<tr>
<td>WB103</td>
<td>IgG1</td>
<td>NS3 (p80) non-structural protein of BVDV</td>
<td>Sopp et al. (1994)</td>
</tr>
<tr>
<td>WB112</td>
<td>IgG2a</td>
<td>NS3 (p80) non-structural protein of BVDV</td>
<td>Sopp et al. (1994)</td>
</tr>
<tr>
<td>TRT-1</td>
<td>IgG1</td>
<td>Turkey rhinotracheitis virus control</td>
<td>Cook et al. (1993)</td>
</tr>
<tr>
<td>TRT-6</td>
<td>IgG2a</td>
<td>Turkey rhinotracheitis virus control</td>
<td>Cook et al. (1993)</td>
</tr>
<tr>
<td>AV29</td>
<td>IgG2b</td>
<td>Chicken CD4</td>
<td>T. F. Davidson (IAH Compton; unpublished)</td>
</tr>
</tbody>
</table>

*WC1 is a 215 kDa antigen on bovine γδ T cells.*
assays or cultured at 2.5 × 10^6 per ml for 3 days. All incubations were at 37 °C in 5% CO₂ in air, unless otherwise stated. Cultured monocytes, adherent on plastic, were removed with 1 ml warmed cell-dissociation fluid (Sigma), washed twice in TCM, resuspended at 10^6 per ml and used in proliferation assays.

**Proliferation assays.** Purified monocytes from the PI calf (PI monocytes), used directly or cultured for 3 days, were irradiated (20 Gy from a 137Cs source) and dilutions were incubated with 10⁵ CD4⁺ or CD8⁺ T lymphocytes from naïve or immune calves. Total volumes were made up to 200 µl with TCM in 96-well U-bottomed microtitre plates (Becton Dickinson). Triplicate cultures were incubated for 5 days and 37 Bq [³H]thymidine ([³H]-Tdr; DuPont) was added for 16 h (overnight) before harvesting. Incorporated radioactivity was determined by liquid scintillation counting. In some experiments, monocytes were purified...
from immune calves and infected by adding BVDV strain Pec515 at an m.o.i. of 2 per monocyte (Brownlie et al., 1984) for 3 days. These cells and uninfected control cells were used as APC with autologous CD4+ or CD8+ T lymphocytes.

**Flow-cytometric analysis.** Two-colour staining of PBMC for leukocyte differentiation antigens and intracellular BVDV NS3 (p80) protein was performed by using a slight modification of the procedure described by Sopp et al. (1994). All samples were diluted and washed in PBS (pH containing 1% BSA and 0.1% sodium azide. Mouse MAbs to bovine CD antigens (Table 1), optimally diluted, were added to 106 PBMC for 10 min at room temperature, washed three times and incubated for 10 min with optimally diluted goat anti-mouse secondary antibodies, conjugated to either FITC or PE (Southern Biotechnology Associates). After three washes, PBMC were fixed in 1% paraformaldehyde (Sigma) in PBS for 10 min. All subsequent washing used PBS/0.1% sodium azide/0.1% saponin (Sigma). After fixation, cells were washed three times and optimally diluted mouse anti-BVDV MAb or an isotype-matched control MAb was added to the samples. Cells were incubated for 10 min, washed and an FITC- or PE-conjugated secondary goat antimouse MAb to a different mouse Ig isotype was added. Immunofluorescent staining was analysed by using a FACScan (Becton Dickinson) and data were analysed by using WinMDI (obtained from Joseph Trotter, Scripp Research Institute, San Diego, CA, USA) and FCS Express (De novo Software).

**Results**

**Flow-cytometric analysis of PBMC from the PI calf**

PBMC isolated from the PI calf were stained and analysed by two-colour flow cytometry in order to establish the percentage of the individual PBMC subpopulations infected with virus (Fig. 1). Expression of the non-structural protein NS3 (p80) indicates that cells are productively infected with BVDV. It was evident that 43±13% of the CD14+ cells (monocytes) stained with a MAb to the BVDV NS3 antigen. Thus, almost half of the monocytes were infected with BVDV. Of the other PBMC subpopulations, 28±7% of CD4+ cells, 31±7% of CD8+ cells, 23±1% of WC1 cells and 23±4% of B cells stained with a MAb to BVDV NS3 antigen. These values represent the means of three separate experiments.

The extents of infection with BVDV for monocytes and lymphocyte subpopulations were similar to previously published values (Sopp et al., 1994). Although the percentages of infected cells in each subpopulation varied from day to day, monocytes always gave the highest percentage of infected cells, with B cells and WC1+ γδ T cell receptor (TCR)+ T cells giving the lowest. After the monocytes had been cultured for 3 days, the percentage that stained with a MAb to BVDV NS3 increased to approximately 90%.

**Monocytes isolated from a calf PI with BVDV (PI monocytes) stimulate the proliferation of resting CD4+ T memory cells**

PI monocytes, freshly isolated (Fig. 2a) or cultured for 3 days (Fig. 2b), stimulated a proliferative response in immune CD4+ T lymphocytes. The proliferative response of the CD4+ T cells to PI monocytes that had been cultured for 3 days was consistently higher (approximately 10-fold) than the proliferative response induced by freshly isolated PI monocytes. CD4+ cells or CD14+ cells cultured alone did not incorporate [3H]thymidine (< 500 c.p.m.). The data shown in Fig. 2 are representative of 14 separate experiments, with CD4+ T cells isolated from two BVDV-immune animals.

**PI monocytes do not stimulate proliferation of CD4+ T cells from BVDV-naive calves**

In order to confirm that the proliferative responses observed were specific for BVDV, CD4+ monocytes from the PI calf were examined for the ability to stimulate proliferative responses in CD4+ T cells isolated from MHC-identical, BVDV-naive calves. Neither freshly isolated nor cultured monocytes stimulated the proliferation of CD4+ T lymphocytes isolated from BVDV-naive animals (Table 2). The number

![Fig. 2](image_url)
of counts obtained for CD4⁺ T cells when cultured with monocytes was not significantly increased above controls for CD4⁺ cells alone and CD14⁺ cells alone, which were <1000 c.p.m. for all experiments. A total of 13 separate experiments was carried out with T cells isolated from three animals.

**CD8⁺ T lymphocytes from calves immune to BVDV are stimulated to proliferate by PI monocytes**

Both freshly isolated PI monocytes (Fig. 3a) and PI monocytes cultured for 3 days (Fig. 3b) stimulated the proliferation of CD8⁺ T cells from BVDV-immune calves. As with the CD4⁺ responses, the cultured PI monocytes consistently induced greater proliferative responses than did the freshly isolated PI monocytes, usually around 10-fold greater. Proliferative responses in CD8⁺ T cell cultures without added monocytes were <500 c.p.m. The results shown are representative of 15 experiments, with CD8⁺ cells isolated from two immune calves.

T cells that express the γδ TCR will undergo a polyclonal proliferative response to irradiated monocytes used as APC in autologous mixed-leukocyte reactions (Hanby-Flarida et al., 1996). In order to determine whether the proliferating CD8⁺ cells were γδ TCR⁺ cells, the percentage of γδ TCR⁺ cells within the CD8⁺ population was determined at the end of the 5 day culture period and compared to the percentage seen at the initiation of the culture.

Flow-cytometric analysis of the CD8⁺ cells after 5 days cultured with the PI monocytes (Fig. 4h) indicated that the γδ TCR⁺ population comprised less than 10% of the total live cells in the wells, indicated by gated region 1 (R1) in Fig. 4(d). γδ TCR⁺ cells also accounted for 11% of the large blast-like cells indicated, by size, in region 2 (R2) (Fig. 4i, m). At time zero, analysis of the PBMC indicated that approximately 4% of the total PBMC were CD8⁺ γδ TCR⁺ (Fig. 4c; upper-right quadrant). This was approximately 28% of the total cells that stained for CD8⁺ antigen. Therefore, the percentage of γδ TCR⁺ cells within the CD8⁺ population decreased with incubation from 28% to <10%. This implied that the proliferative response of CD8⁺ T cells to PI monocytes was due to an expansion of the population of γδ TCR⁺ T cells, indicating a BVDV antigen-specific response. The data presented in Fig. 4 are representative of three separate experiments.

**CD8⁺ T lymphocytes from non-immune (seronegative) calves do not proliferate in response to PI monocytes**

Neither fresh nor cultured PI monocytes stimulated a proliferative response in CD8⁺ T cells isolated from a BVDV-naïve animal. Nine experiments, with CD8⁺ cells isolated from three naïve calves, were undertaken. In none of the experiments was proliferation significantly greater than the controls obtained for the controls of CD8⁺ T cells alone and monocytes alone, which were always <1000 c.p.m. (Table 2).

**CD4⁺ and CD8⁺ T lymphocytes from immune calves proliferate in response to in vitro-infected monocytes**

Monocytes from calves immune to BVDV, cultured with BVDV Pec515 for 3 days, were shown to be infected with BVDV, with over 90% of the monocyte population being NS3⁺ (p80⁺) when assessed by flow cytometry (data not shown). Fig. 5 shows the proliferative response of autologous, purified CD4⁺ and CD8⁺ T lymphocytes to monocytes infected with BVDV or non-infected. The response is similar to that seen with 3-day-cultured PI monocytes and immune CD4⁺ (Fig. 2h) and CD8⁺ (Fig. 3b) T lymphocytes. The data are representative of three separate experiments.

---

**Table 2. Responses of CD4⁺ and CD8⁺ T cells isolated from BVDV-naïve calves to PI monocytes**

Monocytes were isolated from the PI calf by paramagnetic sorting and used as APC either immediately (fresh) or after 3 days in culture (cultured). Values represent the maximum incorporation of [³H]thymidine in c.p.m. for all of the experiments under each set of defined conditions. Numbers in parentheses are the numbers of separate experiments conducted for individual animals.

<table>
<thead>
<tr>
<th>Animal*</th>
<th>CD4⁺</th>
<th>CD8⁺</th>
<th>CD4⁺</th>
<th>CD8⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>6002</td>
<td>&lt; 500 (4)</td>
<td>ND</td>
<td>&lt; 1500 (4)</td>
<td>&lt; 800 (4)</td>
</tr>
<tr>
<td>6111</td>
<td>&lt; 700 (2)</td>
<td>&lt; 700 (2)</td>
<td>&lt; 500 (1)</td>
<td>ND</td>
</tr>
<tr>
<td>7292</td>
<td>&lt; 200 (1)</td>
<td>&lt; 600 (1)</td>
<td>&lt; 200 (1)</td>
<td>&lt; 200 (2)</td>
</tr>
</tbody>
</table>

*Animal from which T cells were isolated.
Mechanisms of virus-induced APC dysregulation are diverse and include the excess production of TGFβ, demonstrated by macrophages infected with African swine fever virus (Whittall & Parkhouse, 1997), blocking of the MHC class I and class II antigen presentation pathways by at least four different proteins encoded within the genomes of herpesviruses and the reduced ability of APC infected by viruses as diverse as measles virus and human immunodeficiency virus to secrete IL-12 (reviewed by Spriggs, 1996; Tortorella et al., 2000). Decreased IL-12 production and secretion affects the ability of the immune system to generate a Th1-type immune response, which is essential for the rapid clearance of virus pathogens.

In vitro studies with BVDV have shown that infection of monocytes or macrophages causes the synthesis of cytokines that may be responsible for the reduced ability to stimulate T cell responses to specific antigens and mitogens. Adler et al. (1996) reported a decrease in the secretion of TNFα from LPS- or Salmonella-stimulated bone marrow-derived macrophages that were infected with either ncp or cp BVDV. Differential priming of monocytes, by ncp or cp BVDV, for nitric oxide production (Adler et al., 1994), the reduction of monocyte responses to chemotactic stimuli (Ketelsen et al., 1979) and production of an inhibitor of IL-1 activity by infected monocytes (Jensen & Schultz, 1991) have all been reported. Thus, it was possible that the specific immunotolerance to BVDV that is evident in PI cattle and is a central component of the pathogenesis of MD is a consequence of infection of APC in vivo.

Making use of cattle of the same MHC haplotype, it was established that ex vivo monocytes from a PI animal were able to stimulate resting CD4+ T memory cells. This implies that the APC have taken up exogenous BVDV antigen, processed that antigen via the endosomal pathway and presented the resultant peptides in association with MHC class II molecules. The response of the CD4+ T cells to BVDV antigen was equivalent to that seen in other antigen-specific systems (Knight & Macatonia, 1991; Schlesier et al., 1994) and is similar to that reported by Rhodes et al. (1999).

Monocytes from a PI calf, cultured for 3 days, were more effective than fresh monocytes at inducing proliferative responses of memory T cells. This could be for a number of reasons. There was an increase in the number of infected cells after 3 days in culture, as almost 90% of the monocytes stained with MAb to the NS3 (p80) antigen whereas only approximately 43% of the freshly isolated monocytes stained. Additionally, placing monocytes into culture is known to cause a transitory increase in the ability of the cells to stimulate T cells and a corresponding upregulation of co-stimulatory and adhesion molecules, before full differentiation into macrophages, which display poor accessory cell function (Mayernik et al., 1983; Najar et al., 1990). It is likely that the combination of an increase in APC function, due to culture, and an increase in the number of infected cells that present viral antigen is responsible for the increase in T cell responses to cultured PI monocytes when compared with ex vivo PI monocytes. Thus, APC infected in vivo and isolated from the PI animal and APC cultured for 3 days, which were a mixture of in vivo- and in vitro-infected cells, did not appear to be compromised in their ability to present BVDV antigen to CD4+ T cells and to stimulate a MHC class II-restricted T cell response.

PI monocytes were also able to stimulate a CD8+ MHC class I-restricted T cell response in CD8+ T cells isolated from BVDV-immune cattle. As with the CD4+ T cell response, 3-day-cultured monocytes stimulated a 10-fold higher pro-
Fig. 4. Analysis of cultures for CD8⁺ γδ TCR⁺ T cells. Cells were stained at time zero (a–c) and after 5 days of incubation (d–m). (a) R1 gate indicates live cells in PBMC isolated from the PI calf. These cells were analysed further in (b) and (c). (b) Staining with control MAAb on R1 cells. (c) Percentages of cells stained with MAabs to CD8 (13%, vertical axis) and γδ TCR (25%, horizontal axis). Cells that were CD8⁺ and γδ TCR⁺ (4%) are located in the top-right quadrant. (d)–(h) Percentages of live cells that were CD8⁺ (f) or γδTCR⁺ (h) in the proliferating CD8⁺ T cell population (R1 in d) after 5 days of culture with PI monocytes are indicated. (i)–(m) Percentages of large, blast-like cells within the culture that were CD8⁺ (k) or γδ TCR⁺ (m) are indicated. Approximately 28% of the CD8⁺ T cells in the initial PBMC population were γδ TCR⁺ (c). After 5 days of culture with PI monocytes, approximately 10% of the CD8⁺ population expressed γδ TCR (h, m), indicating that γδ TCR⁺ CD8⁺ T cells did not proliferate in response to the PI monocytes.
E. J. Glew and C. J. Howard

![Graph](Image)

Fig. 5. Monocytes infected in vitro with BVDV stimulate CD4\(^+\) and CD8\(^+\) T lymphocyte responses. Monocytes were isolated from an immune calf that was not PI and were infected with BVDV by culturing for 3 days with virus at an m.o.i. of 2. Varying numbers of infected monocytes (filled bars) were incubated with \(10^5\) CD4\(^+\) (a) or CD8\(^+\) (b) T lymphocytes as in Figs 2 and 3. Open bars indicate T cells incubated with non-infected monocytes. The data are representative of three experiments.

Proliferative response in CD8\(^+\) T cells than did the freshly isolated monocytes. Cross-presentation is a feature of dendritic cells and is a means by which non-replicating antigen, normally processed by the exogenous pathway and presented in the context of MHC class II molecules, is presented by MHC class I molecules with the subsequent induction of CD8\(^+\) T cell responses (Grommé et al., 1999). However, this pathway has not been reported for monocytes. Thus, the CD8\(^+\) T cell responses demonstrated here would be expected to be directed against viral antigen, processed via the endogenous pathway only and presented in association with MHC class I. The APC used here were infected naturally in vivo for the freshly isolated cells, or were a mixture of in vitro- and in vivo-infected cells when the APC were cultured for 3 days. These results suggest that APC from the PI animal were not compromised in their ability to stimulate an MHC class I-restricted T cell response and that BVDV does not exert a suppressive effect on the endogenous pathway of antigen processing.

Furthermore, the proliferative response of CD8\(^+\) as well as CD4\(^+\) T cells from immune calves to 3-day-cultured PI monocytes was similar to that noted with monocytes that were obtained from an immune calf that was not PI and which had been infected in vitro by incubation for 3 days with BVDV. This indicates that PI monocytes do not differ from ‘normal’ monocytes in their capacity to differentiate into potent APC. The lack of a response with T cells from naive animals confirmed that neither recognition of major or minor histocompatibility antigens by the responding T cells nor a non-specific effect of the virus on the APC was responsible for major component of the response seen.

Previous studies, from experiments that involved depletion of CD4\(^+\) and CD8\(^+\) T cells in vivo (Howard et al., 1992), and the observation that passive antibody can protect against transient, acute BVDV infection (Howard et al., 1989) have been taken to indicate that CD4\(^+\) T cells and antibody, and not cytolytic CD8\(^+\) T cells, are the main component in the recovery from and immunity of animals to BVDV. This mechanism has also been demonstrated for mice infected with lymphocytic choriomeningitis virus (Planz et al., 1997). However, a critical role for CD8\(^+\) T cells in the immune response should not be ruled out. The results presented here show that a CD8 memory T cell response is evident in previously infected cattle. Furthermore, shedding of BVDV in nasal secretions has been noted that persisted in the presence of serum neutralizing antibodies (Fray et al., 1998; Howard et al., 1999) and different effector cell populations may have varying degrees of importance in different tissues, as suggested for a murine gammaherpesvirus (Ehtisham et al., 1993).

References


Received 24 January 2001; Accepted 14 March 2001