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Immunity to bovine virus diarrhoea virus in calves: the role of different T-cell subpopulations analysed by specific depletion in vivo with monoclonal antibodies

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ABSTRACT

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Gnotobiotic calves were injected intravenously with murine monoclonal antibodies (mAb) directed against the BoCD4, BoCD8 or BoWC1 antigens that define the three major T-lymphocyte subpopulations in cattle. This produced a transient, specific depletion of each cell type in the circulation. Calves were then infected intranasally with a non-cytopathogenic biotype of bovine virus diarrhoea virus and the effect of the specific depletion with the mAb on viraemia and shedding of virus from the nasopharnyx determined. Depletion of the cells expressing the BoCD4 antigen resulted in an extension of the duration of viraemia and an increase in the titre of virus in blood. No effect on nasopharyngeal shedding was noted. Depletion of either of the other two T-cell subsets that expressed the BoCD8 antigen or the BoWC1 antigen present on the gamma/delta T-cells had no demonstrable effect. These findings are interpreted as showing that the BoCD4$^+$ cells play a pivotal role in controlling a primary infection with this virus but MHC class I restricted BoCD8$^+$ T-cells are not a major effector mechanism. The BoCD4$^+$ cells may be acting directly or be mediators of T-cell help.

ABBREVIATIONS

BoWC1, Bovine Workshop Cluster 1; BVDV, bovine virus diarrhoea virus; i.v., intravenous; mAb, monoclonal antibodies; nc, non-cytopathogenic; PBMC, peripheral blood mononuclear cells.

INTRODUCTION

Bovine virus diarrhoea virus (BVDV) infections in cattle can range from the mild clinically inapparent to the fatal mucosal disease (Brownlie, 1990).

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Mucosal disease has a complex pathogenesis and is now known to be a consequence of foetal infection with the non-cytopathogenic biotype and a superinfection later in life with the cytopathogenic biotype of the same virus (Brownlie et al., 1984). Preventing infection of the bovine foetus is the key to controlling the diseases caused by BVDV and would be economically beneficial in reducing mortality and morbidity in young animals reared for beef as well as in breaking the cycle leading to mucosal disease.

Several reports have shown that specific antibody is an important mediator of immunity to BVDV and can control infection resulting from both systemic and respiratory challenge (House and Manley, 1973; Shope et al., 1976; Howard et al., 1989a). Although immunity to reinfection can be mediated by antibody it is possible that cytotoxic T-cells are an important effector mechanism in the recovery from primary virus infection. Classically, this question has been answered for several virus infections in rodents by passively transferring cells between inbred animals. Such experiments are not presently possible in outbred domestic animals, but may subsequently be answered by using progeny derived from embryo splitting and cloning techniques. An alternative method is to specifically deplete lymphocyte subpopulations in vivo by utilising monoclonal antibodies (mAb) specific for T-cell subpopulations. Investigations of this nature have been used to great effect in rodents to analyse the immune response to a variety of inert antigens and infectious microbes (Waldman, 1989). It has also been shown that murine mAb, injected intravenously, will specifically deplete T-cell subpopulations in calves and that this has significant immunological effects (Howard et al., 1989b).

The major T-lymphocyte subpopulations in cattle can be defined by their expression of one of three surface molecules. MHC class I restricted T-cells synthesize the BoCD8 molecule (Ellis et al., 1986) which is the bovine homologue of CD8 in humans; these cells are the classical cytotoxic T-cells. MHC class II restricted T-cells synthesise the BoCD4 molecule (Baldwin et al., 1986); these cells were originally considered, from functional data, to be the T-helper population. A third T-cell population in cattle, that comprises some 25% of peripheral blood mononuclear cells (PBMC) in young calves, can be identified with mAb to a 215/300 kDa surface molecule named Bovine Workshop Cluster 1 (BoWC1) (Howard and Morrison, 1991). These cells synthesise the gamma/delta T-cell receptor and are BoCD2$^-$, BoCD4$^-$, BoCD8$^-$ (Clevers et al., 1990). The role of this T-lymphocyte subpopulation is the subject of much current investigation.

In this report we describe experiments in which calves were depleted of these three subpopulations of T-cells and the effect of this depletion on infection with BVDV.

MATERIALS AND METHODS

Bovine virus diarrhoea virus

A non-cytopathogenic (nc) strain of BVDV, Pe515nc (Brownlie et al., 1984) was cultured in calf testis cells and stored at −70°C to provide a standard inoculum of 5×10^6 TCD_{50} per calf. Methods for the isolation and quantitation of infectious virus in blood or from nasopharyngeal swabs have been described previously (Howard et al., 1989a).

Monoclonal antibodies

mAb are listed in Table 1. In vivo depletion following intravenous (i.v.) injection of mAb was assayed as described previously (Howard et al., 1989b). Calves were injected i.v. with 4 mg doses of murine Ig as noted below. The mAb used to deplete were: CC8, anti-BoCD4; CC63, anti-BoCD8; CC38, anti-BoCD6 which is present on the majority of $CD2^+$, $CD4^+$, $CD8^+$ T-cells in bovine PBMC (Baldwin et al., 1988); CC15, anti-BoWC1 which is present on $CD2^-$, $CD4^-$, $CD8^-$, $CD5^+$ T-cells that synthesise the gamma/delta T-cell receptor (Clevers et al., 1990). These and the other mAb listed (Table 1) were used to stain PBMC that had been isolated from blood collected into heparin (10 U ml^{-1}) and fractionated on Histopaque 1083 (Sigma, Poole, Dorset, UK). The cells were analysed by flow cytometry on a FACScan (Becton Dickinson, Mountain View, California, USA) by the methods previously reported (Howard et al., 1988, 1989b) enabling the quantitation of B-cells, T-cells and monocytes in PBMC.

TABLE 1

Specificity of monoclonal antibodies

mAb	Specificity	Reference
CC42	BoCD2	Davis and Splitter, 1991
CC8	BoCD4	Bensaid and Hadlam, 1991
CC17	BoCD5	Howard et al., 1988
CC38	BoCD6	Letesson and Bensaid, 1991
CC63	BoCD8	MacHugh and Sopp, 1991
CC15	BoWC1 ($CD4^-$, $CD8^-$ T-cells)	Clevers et al., 1990
CC21	BoWC3 (B-cells)	Naessens et al., 1990
IL-A30	IgM	Naessens et al., 1988
IL-A24	Monocytes/granulocytes	Ellis et al., 1988b

Animals

Friesian cross Aberdeen Angus calves were derived by caesarean section and maintained in a microbial free environment in flexible plastic isolators (Dennis et al., 1976). Calves were shown to be free of BVDV and antibody and thus not to have been exposed to the virus in utero.

Serological assays

Antibody to BVDV was measured by ELISA (Howard et al., 1985). Antibody directed against murine IgG was measured by ELISA using IgG2a purified on Sepharose Protein-A (Pharmacia, Milton Keynes, UK) from ascitic fluid derived from mice injected with a hybridoma that secreted an mAb (code number CC4) of unrelated specificity to those used for injection into calves.

Experimental protocols

Virus (5 ml of suspension) was inoculated intranasally into calves when between 24 and 52 days of age, designated Day 0. Nasopharyngeal swabs and blood for virus isolations were collected on Days 0, 3, 5, 6, 7, 10, 14, 21 and 28. Leukocyte quantitation and phenotyping was also done on these days as well as Days −2 and 12. Serum was collected on Days −2, 7, 14, 21, 28 and 42. mAb were injected i.v. on Days −2, 0, 3, 5, 7 and 10 for calves numbered 4 and 56. The remaining calves were injected with mAb daily from Days 0 to 4. Three calves received BoCD4, two BoCD8, two BoWC1, two BoCD4 plus BoCD8 plus BoCD6 mAb and three were untreated. One millilitre of Finadyne (Schering-Plough, Mildenhall, UK) was injected i.v. on the day when mAb were first administered.

RESULTS

Effect of mAb administration

A small fall in the number of mononuclear leukocytes followed inoculation with mAb that corresponded to the proportion of the target cell population in PBMC. No adverse effects were seen following the initial injections of mAb CC8 (BoCD4) but a brief period, during which the respiratory rate of the calves was raised, was noted following the injection of mAb on Day 10 indicating that an anaphylactic response had been generated. For this reason the protocol was subsequently changed to daily injections of mAb for 5 days and no similar problems arose.

Specific depletion of the appropriate T-lymphocyte subpopulation was evident in calves that had been injected with mAb (Table 2). Thus, in calves

TABLE 2

Effect of injecting mAb on the lymphocyte composition of blood

Treatment[1] group	mAb used to stain PBM	Percentage[2] of cells in PBM staining with mAb on Day:						
		0	3	5	7	14	21	28
BoCD4	BoCD4	28	3	1	1	6	7	11
	BoCD8	13	13	15	15	22	25	26
	BoWC1	16	27	31	32	25	24	24
BoCD8	BoCD4	39	24	35	37	43	39	42
	BoCD8	15	<1	6	6	7	8	7
	BoWC1	21	27	26	15	14	18	17
BoWC1	BoCD4	28	20	42	32	24	35	40
	BoCD8	15	18	21	17	20	22	26
	BoWC1	27	1	<1	<1	4	5	6
BoCD4 + BoCD8 + BoCD6	BoCD4	35	6	3	1	10	12	18
	BoCD8	14	5	4	7	12	15	16
	BoWC1	20	34	42	37	40	37	35
	BoCD6	51	2	1	4	26	24	34

[1]mAb inoculated indicated; mAb CC8 (BoCD4) injected into Calves 4, 56, 173; mAb CC63 (BoCD8) injected into Calves 196, 198; mAb CC15 (BoWCI) injected into Calves 256, 260; mAbs CC8, CC63, CC38 (BoCD6) injected into Calves 391. 395.
[2]Average values of percentage of PBM staining with indicated mAb given.

TABLE 3

Antibody response of calves to mouse IgG2a

Treatment group[1]	Titre[2] by ELISA on Week:					
	0	1	2	3	4	6
Control	–	–	–	–	–	–
BoCD4	–	–	1.7	2.5	2.6	3.2
BoCD8	–	1.5	4.1	4.3	4.5	4.2
BoWC1	–	–	3.1	2.9	3.0	3.0

[1]BVDV injected into all calves; mAb injected as indicated.
[2]Log_{10} reciprocal of highest dilution of serum greater than twice background; geometric mean for treatment group given; –, no specific antibody detected, titre $<10^{1.3}$.

injected with mAb to BoCD4 the number of cells expressing this antigen fell from an average of 28% to a minimum of 1%. In calves injected with mAb to BoCD8 the percentage of $BoCD8^+$ cells fell from 12% and 17% to <1%. Calves injected with mAb directed against the BoWC1 antigen showed a fall in $BoWC1^+$ cells to <1% from pre-injection values of 26% and 28%. The BoCD8 cells appeared to return more rapidly than the others and 5 days after the first injection of the mAb 6% of PBMC were $BoCD8^+$; in contrast, after

injection of mAb directed against BoCD4 to BoWC1 the number of the cells that expressed either of these antigens remained at $< 1\%$ for at least 7 days.

An antibody response directed against constant regions on the IgG2a molecule was evident in all of the calves that had been injected with murine mAb irrespective of their specificities (Table 3).

Effect of infection with BVDV on the lymphocyte composition of PBMC

Intranasal infection of gnotobiotic calves with BVDV caused a slight transient leukopenia. In animals infected with virus but not injected with mAb the percentage of BoCD4$^+$ T-cells fell by a maximum of 6%, the number of BoCD8$^+$ T-cells fell by 3% and the number of BoWC1$^+$ T-cells did not fall (Table 4). These trends were not statistically significant but consistent with the finding was the observation that animals injected with mAb to BoCD8 or BoWC1 also showed a fall in the percentage of BoCD4$^+$ cells (Table 2) whereas there was a slight increase in the other T-cell phenotype that appeared to compensate for the specific depletion due to the mAb. Calves injected with mAb CC15 (BoWC1) but not injected with BVDV did not show a fall in the number of BoCD4$^+$ cells (Howard et al., 1989b).

Effect of depletion with mAb on infection with BVDV

The days on which virus was isolated from blood and from the nasopharynx, together with the titre of virus in these samples is given in Tables 5 and 6. Duration of viraemia was 2–9 days in control calves and the peak mean virus titre was $10^{1.9}$. Viraemia was prolonged in calves that had been treated

TABLE 4

Effect of BVDV on the lymphocyte composition of blood

Antigen assessed	Percentage[1] of cells in PBMC stained on Day:						
	0	3	5	7	14	21	28
IgM (B-cells)	11 ±2.1	15 ±6.0	9 ±2.0	9 ±6.8	10 ±5.0	9 ±4.7	13 ±8.5
BoCD2	49 ±2.9	39 ±9.6	45 ±6.7	35 ±4.0	47 ±3.2	44 ±3.9	51 ±4.2
BoCD4	21 ±5.5	18 ±8.4	18 ±5.0	15 ±6.0	20 ±4.6	21 ±2.6	23 ±4.2
BoCD8	13 ±2.6	11 ±3.2	11 ±3.5	10 ±4.0	16 ±3.0	16 ±2.6	15 ±3.5
BoWC1	32 ±4.7	39 ±5.0	41 ±2.1	31 ±12.5	31 ±4.2	30 ±4.6	30 ±7.8

[1]Mean ± SD for three calves.

TABLE 5

Effect in calves of depletion of lymphocyte subpopulations on viraemia with BVDV

Treatment[1] group	Calf No.	Age (days)	Day								
			0	3	5	6	7	10	14	21	28
Controls	211	52	–[2]	–	1.0[3]	0.7	1.2	–	–	–	–
	52	31	–	–	–	1.7	1.7	1.5	1.0	–	–
	172	30	–	–	–	2.2	1.0	–	–	–	–
BoCD4	4	38	–	1.2	2.2	3.2	2.7	1.0	1.5	1.5	–
	56	24	–	–	1.2	2.2	2.7	1.7	1.2	–	–
	173	29	–	–	1.7	2.2	1.7	1.5	1.0	–	–
BoCD8	196	32	–	–	1.0	1.0	1.5	–	–	–	–
	198	29	–	–	1.0	1.5	1.7	1.0	–	–	–
BoWC1	256	31	–	–	–	1.0	1.5	–	–	–	–
	260	24	–	–	1.0	1.2	1.5	–	–	–	–
BoCD4+	391	37	–	–	1.5	1.2	1.5	1.0	1.0	–	–
BoCD8+ BoCD6	395	36	–	–	–	–	1.5	2.2	1.5	–	–

[1]mAb inoculated as for Table 2.
[2]–, <0.5 $\log_{10}$ TCD_{50} ml^{-1}.
[3]$\log_{10}$ TCD_{50} ml^{-1} blood.

TABLE 6

Effect of depletion with mAb on the isolation of BVDV from the nasopharynx of calves

Treatment[1] group	Calf No.	Day								
		0	3	5	6	7	10	14	21	28
Controls	211	–[2]	–	0.7[3]	1.2	–	–	–	–	–
	52	–	–	–	1.7	1.7	–	–	–	–
	172	–	–	–	0.7	–	–	–	–	–
BoCD4	4	–	–	1.7	–	1.7	–	–	–	–
	56	–	–	0.7	1.7	1.7	–	–	–	–
	173	–	–	1.7	1.7	–	–	–	–	–
BoCD8	196	–	–	1.7	–	0.7	–	–	–	–
	198	–	–	–	–	–	–	–	–	–
BoWC1	256	–	–	–	–	1.2	–	–	–	–
	260	–	–	0.7	0.7	1.7	–	–	–	–
BoCD4+	391	–	1.2	1.2	–	1.2	0.7	–	–	–
BoCD8+ BoCD6	395	–	–	1.7	–	2.7	3.2	3.2	1.7	–

[1]mAb inoculated as for Table 2.
[2]–, <0.2 $\log_{10}$ TCD_{50} ml^{-1}.
[3]$\log_{10}$ TCD_{50} ml^{-1} in nasopharyngeal swab.

TABLE 7

Effect of depletion with mAb on the antibody response to BVDV

Treatment group	Titre by ELISA on Week:					
	0	1	2	3	4	6
Control	1.2[1] ±0.05	1.2 ±0.08	1.2 ±0.06	1.5 ±0.62	1.9 ±0.72	2.7 ±0.45
BoCD4	1.5 ±0.55	1.2 ±0.05	1.3 ±0.02	1.2 ±0.02	2.0 ±0.74	2.8 ±0.24
BoCD8	1.2 ±0.01	1.2 ±0.01	1.2 ±0	1.6 ±0.80	2.5 ±0.05	3.0 ±0.02
BoWC1	1.2 ±0.16	1.2 ±0.13	1.3 ±0.03	1.7 ±0.77	2.4 ±0.18	2.8 ±0.30

[1]Log_{10} number of units per millilitre in serum, mean ± SD.

with mAb to the BoCD4$^+$ lymphocytes and was of 10–19 days duration, $P<0.05$ by analysis of variance compared to controls. The virus titre in blood was also greater in the mAb-treated calves compared to controls, $P<0.01$. In contrast, depletion of the BoCD8$^+$ or BoWC1$^+$ subpopulations had no demonstrable effect on either the duration of viraemia or the titre of virus in blood. In calves that had been treated with mAb directed against the BoCD4, BoCD8 and BoCD6 antigens no additional effect over that due to treatment with mAb to BoCD4 alone was evident.

Treatment with mAb to deplete the BoCD4$^+$, BoCD8$^+$ or BoWC1$^+$ subpopulations was not demonstrated to have any effect on the duration of nasopharyngeal shedding or the titre of virus in swabs. In calves that had been treated with mAb to the BoCD4, BoCD8 and BoCD6 antigens duration of shedding was extended to 8–17 days from the 1–2 days in the control calves.

Antibody to BVDV in infected calves

Control calves and calves treated with mAb produced similar antibody responses (Table 7).

DISCUSSION

Intranasal inoculation of BVDV into immunocompetent gnotobiotic calves resulted in a transient viraemia and period of nasopharyngeal shedding of virus similar to that observed in conventionally reared calves (Howard et al., 1989a). An interesting aspect of this infection was the possibility that the BoCD4$^+$ T-cells appeared to be affected more than the BoCD8$^+$ or BoWC1$^+$ (gamma/delta) T-cells. Such an effect in vivo, even if transient, could have a significant consequence for the immune response of calves to other agents

and explain the immunosuppressive phenomena long associated with BVDV infections (Reggiardo and Kaeberle, 1981; Yates, 1982; Potgieter et al., 1984). Other studies on the effect of acute infection of normal immunocompetent calves with BVDV broadly agree with the observations made here but differ in some of the details. Bolin et al. (1985) showed that after intravenous challenge with a cytopathogenic strain of BVDV the number of both T- and B-cells was decreased but the T-cells were more severely affected. Ellis et al. (1988a) 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 gamma/delta T-cell receptor were shown. Differences in the strain of virus used, the dose and biotype or age of cattle could affect individual observations. However, preliminary investigations have not shown any effect of depletion on pathogenicity due to a cytopathogenic BVDV strain (C.J. Howard, M.C. Clarke, P. Sopp and J. Brownlie, unpublished data, 1980). Further comparisons are required to define the variable pathogenicity of different strains and their interaction with, and effect on, the immune system.

In the results reported here the injection of mAb into calves resulted in the specific depletion of the appropriate T-cell subpopulation. Depletion of the BoCD4$^+$ cells resulted in increased susceptibility to infection but depletion of either the BoCD8$^+$ cells or the BoWC1$^+$ gamma/delta T-cells alone had no observable effect. We therefore conclude that the BoCD4$^+$ T-cells play a major role in recovery from primary infection with BVDV. This could either be the result of helper activity for Ig production, from the production of cytokines involved in activation of accessory cells or be due to the generation of MHC class II restricted cytotoxic T-cells with none of these possibilities being exclusive. No evidence was obtained to indicate that MHC class I restricted cytotoxic T-cells are the major effector cell in recovery from primary infection. It appeared more difficult to deplete the BoCD8$^+$ cells than the BoCD4$^+$ cells, the former population reappeared more rapidly than the latter. Nevertheless, a marked reduction in peripheral blood was achieved. The relative insensitivity of BoCD8 cells to depletion may be due to: differences in the mAb such as affinity, differences in complement activation or binding to Fc receptors, differences in the sensitivity of T-cell subpopulations expressing either the BoCD4 or BoCD8 antigens to complement lysis, variations in the number of target molecules on the cell surface or differences in the rate at which different subpopulations re-seed the blood following their removal. The BoWC1$^+$ cells were depleted most effectively; this had no demonstrable effect on viral infection thus no role for this T-cell population in resistance to BVDV infection was established. Interestingly, these cells were the only one of the three major T-cell subsets found to be reduced in persistently viraemic

calves (Howard, 1990). The relation between depletion in the blood and depletion in tissues was not established from the studies reported. Subsequent observations have shown the peripheral nodes and spleen to be markedly affected by the intravenous injection of mAb but further comparisons of this nature are needed to establish whether i.v. injection of mAb would affect lymphoid tissues at mucosal sites and thus viral replication there.

In vivo depletions with mAb have been used previously in rodents to analyse the functional role of different lymphocyte subsets in antiviral and antibacterial immunity and in the pathogenesis of the disease produced. Depletion of the CD4 cells in mice has been shown to affect the outcome of infection due to loss of T-cell help for antibody responses (Nash et al., 1987; Welsh et al., 1987) and activation of macrophages (Muller et al., 1987). Cytotoxic CD8 T-cell responses can remain functional and do not necessarily require CD4 T-cell help (Buller et al., 1987; Nash et al., 1987). Specific depletion of the CD8 cells in mice resulted in an enhanced infection with herpes simplex virus (Nash et al., 1987), *Listeria monocytogenes* (Czuprynski and Brown, 1990) and *Leishmania major* (Titus et al., 1987) but reduced disease due to lymphocytic choriomeningitis virus due to the role of MHC class I restricted T-cells in the pathogenesis of the disease caused by this virus (Leist et al., 1987).

Depletion of BoCD4$^+$ T-cells in calves reduced their ability to resolve a primary BVDV infection. One possibility is that this was because of an effect on CD4 help for antibody response, which as noted above has been shown to mediate immunity to infection with this virus. However, the depleted calves produced an antibody response similar to that of controls; furthermore, it should be pointed out that the antibody response to BVDV is generally not evident in sera until 3 weeks post infection and the effect of lymphocyte depletion was noted before this. It therefore seems unlikely that antibody is paramount in mediating control of infection at this time after exposure to virus, although it clearly mediates resistance to reinfection subsequently. Depletion of CD8 T-cells was not shown to have an effect on infection, thus the role of the CD4$^+$ cells is presumed not to be via help in the generation of cytotoxic CD8$^+$ T-cells. The role of the CD4 cell could be in the activation of monocytes or macrophages as well as the activation of NK cells. Alternatively, cytotoxic CD4$^+$ T-cells that are specific for BVDV infected target cells may play a critical role in recovery from primary BVDV infection. A CD4$^+$ cytotoxic T-cell clone that recognised plasmodial antigen in the context of MHC class II has been shown to passively protect mice against *Plasmodium berghei* (Tsuji et al., 1990). Further studies should establish the mechanism by which the CD4$^+$ cells operate.

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