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The effect of dexamethasone-induced immunosuppression on the development of faecal antibody and recovery from and resistance to rotavirus infection

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ABSTRACT

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Rotavirus-naive and rotavirus-immune gnotobiotic calves were treated with high doses of dexamethasone (DX) to suppress the immune system. Calves were then infected with a virulent rotavirus inoculum, J-160, to investigate the role of immune responses both in recovery from primary rotavirus infection and in resistance to secondary rotavirus infection. Treatment of calves with DX markedly suppressed in vitro responsiveness of peripheral blood lymphocytes to mitogens within 48 h of the start of DX treatment. Suppression was similar in rotavirus-naive and rotavirus-immune calves. In contrast, the effect of DX treatment on specific antibody responses differed depending on when DX treatment started in relation to rotavirus infection. When DX treatment commenced prior to primary rotavirus infection both systemic and local specific antibody responses were inhibited. These calves, in which mitogen and antibody responses were suppressed, exhibited greater clinical signs than did control calves after infection with virulent rotavirus, but virus excretion was affected in only one of the two calves. When DX treatment was started after primary rotavirus infection but before secondary infection, systemic and local antibody responses to the primary infection and to the challenge infection were not affected. These calves resisted challenge with virulent virus as did DX-untreated rotavirus-immune calves, even though mitogen responses were suppressed.

We conclude that in a primary rotavirus infection, virus excretion ceased when both antibody and mitogen responses were suppressed. Resistance to secondary rotavirus infection occurred when mitogen responsiveness was suppressed, but when antibody levels were normal. Thus, no evidence was obtained that fully functional cell-mediated immune mechanisms are essential for resistance to rotavirus infection. Evidence was provided for the ability of parenteral treatment with DX to suppress mucosal as well as systemic antibody responses.

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ABBREVIATIONS

ConA, concanavalin A; DX, dexamethasone; FCS, fetal calf serum; GC, glucocorticosteroids; [^{3}H]TdR, [^{3}H]thymidine; PHA, phytohaemagglutinin; PWM, pokeweed mitogen; SCID, severe combined immunodeficiency disease; TCID, tissue culture infectious doses.

INTRODUCTION

Following rotavirus infection, systemic and local immune responses are stimulated, including neutralising and non-neutralising antibody (Van Zaane et al., 1986; Saif, 1987) and cell-mediated immunity (Kohl et al., 1983; Totterdell et al., 1988; Offit and Dudzik, 1989). However, it is unclear which of these immune mechanisms are involved either in resolution of primary infections or in resistance of immune animals to secondary rotavirus infections.

Studies of immunodeficient patients with chronic rotavirus excretion and diarrhoea (Saulsbury et al., 1980; Wood et al., 1988) and mice with severe combined immunodeficiency disease (SCID), which persistently excreted rotavirus following experimental infection (Riepenhoff-Talty et al., 1987), indicated the involvement of immune mechanisms in recovery from primary rotavirus infection. However, other studies with T-cell-deficient mice demonstrated recovery from rotavirus infection in the absence of immune responses (Eiden et al., 1986).

The active immune mechanisms which protect the gut from rotavirus disease are not clearly defined. One mechanism which has been thought to be essential is neutralising antibody, but studies in experimental animals and vaccination studies in humans suggest that other mechanisms are important (Woode et al., 1978; Bridger and Oldham, 1987; Clarke et al., 1988).

While the immune system of cattle is considered to be relatively resistant to the effects of glucocorticosteroids (GC) compared to that of mice (Wilkie et al., 1979; Pruett et al., 1987), GC treatment nevertheless has marked effects on bovine immune responsiveness (reviewed by Roth and Kaeberle, 1982). These effects include the suppression of both antibody and cell-mediated immune responses. However, nothing is known of the effects of GC on intestinal antibody responses. In this paper we have used DX-induced immunosuppression to try to gain an understanding of the immune mechanisms involved in recovery from primary enteric infection and resistance to secondary enteric infection. In addition, we report the effect of DX treatment on faecal antibody responses to rotavirus in cattle.

MATERIALS AND METHODS

Viruses

Rotavirus C3-160 was obtained from a healthy calf and had been passaged and cloned in cell culture followed by serial passage in four gnotobiotic calves

(Bridger and Pocock, 1986; Pocock, 1987). The challenge rotavirus inoculum (J-160) was uncloned and has been described previously (faecal CP-l in Bridger and Pocock, 1986). Inocula were prepared as 0.45 μm faecal filtrates.

Animals and experimental design

Gnotobiotic calves were derived (Hoare et al., 1976) and reared on a milk-based diet (Dennis et al., 1976) as previously described. On Day 0, four calves, aged 7 days, were infected with 10^6 $TCID_{50}$ of rotavirus C3-160 (calves B110 and B150 in Group C and calves B180 and C43 in Group D). On Day 13, these four calves and five other rotavirus-naive calves (B111, B130 and C1 in Group A and C2 and C44 in Group B) aged 14–20 days were challenged with 10^5 $TCID_{50}$ of the virulent challenge inoculum J-160. Daily treatment with dexamethasone (DX) (0.5 mg kg^{-1} body weight; Intervet) by i.m. injection was started for the calves in Groups B and D 5 days prior to challenge infection. The experimental design is summarised in Table 1.

Faecal samples were collected daily. With male calves, the daily output was collected with the aid of a harness and collection bag. Sera were obtained every 2 or 3 days.

Disease assessment

A clinical score was obtained by totalling the number of days on which the following clinical signs were observed: change in demeanour; anorexia (de-

TABLE 1

Summary of experimental design[1]

Group	Calves	Rotavirus C3-160 (Day 0)	DX treatment (Days 8–27)	Rotavirus J-160 (Day 13)
A	B111, B130, C1	–	–	+
B	C2, C44	–	+	+
C	B110, B150	+	–	+
D	B180, C43	+	+	+

[1]Gnotobiotic calves were divided into four groups. Calves in Groups C and D were infected with rotavirus C3-160 at 7 days of age (Day 0). On Day 13 calves in all four groups were challenged with virulent virus inoculum J-160. Calves in Groups B and D received daily intra-muscular injections of DX (0.5 mg kg^{-1} body weight) from Day 8 until the end of the experiment (Day 27).

fined as failure to consume food within 60 min of being offered; calves normally drank within 5–10 min); faecal output greater than 500 g day^{-1} (healthy gnotobiotic calves produced 320 ± 80 g day^{-1}); change in faecal colour to light yellow from brown; faecal dry matter content less than 10%; increased packed cell volumes (above preinfection level); and rectal temperature above 39.2 °C.

Virus detection

Virus was detected by infectivity assays conducted in duplicate (Bridger and Pocock, 1986). Titres were expressed as $\log_{10}$ $TCID_{50}$ g^{-1} of faeces or ml^{-1} of culture fluid.

Antibody detection in serum and faeces

Class-specific ELISAs were used to measure antibodies to rotavirus group antigens in serum and faeces as previously described (Bridger and Oldham, 1987) except that isotype-specific monoclonal antibodies (generous gifts from Dr. Naessens, ILRAD, Kenya, anti-IgM and IgG1; and Dr. C. Stokes, Bristol Vet. Med. School, UK, anti-IgA) were used in place of polyclonal antisera. Serum (for IgG1 assay) or faecal extract (for IgM and IgA assays) standards were included in every assay. The absorbance of the test samples was calculated as a percentage of that of the standard. The results shown are the mean of duplicate assays carried out on different days.

From preliminary studies with known negative sera and faecal samples, test samples were considered to be positive when their antibody titres exceeded 10% of the standard for serum and faecal IgM, 5% for serum IgG1, 10% for faecal IgG1, 10% for serum IgA, 20% for faecal IgA.

Lymphocyte proliferation assays to mitogens

Peripheral blood mononuclear cells were isolated by centrifugation of heparinised blood over Histopaque 1083 (Sigma) at $900 \times g$ for 40 min. The cells from the interface were washed three times with PBS and resuspended in tissue culture medium at a concentration of 2×10^6 cells ml^{-1}. The tissue culture medium used was RPMI-1640 (Gibco) supplemented with 10% foetal calf serum (FCS; heat inactivated at 56 °C, 30 min; Flow), 2 mmol glutamine, 100 IU ml^{-1} penicillin, 0.2 mg ml^{-1} streptomycin, 20 mmol HEPES and 27 mmol sodium bicarbonate.

Mononuclear cell preparations (0.2 ml $well^{-1}$, 2×10^6 cells ml^{-1}) were cultured in flat-bottomed microplates (Nunclon Delta, Nunc) with or without the addition of mitogens. Optimal concentrations of the mitogens phytohaemagglutinin (PHA), concanavalin A (ConA) and pokeweed mitogen (PWM) were determined in previous experiments to be 45 μg ml^{-1}, 5 μg ml^{-1} and

1 : 100 of stock respectively. After 72 h culture in a humidified atmosphere of 5% CO_2, in air, 1 μCi [^{3}H]thymidine ([^{3}H]TdR, 5 Ci mmol^{-1}, Radiochemical Centre, Amersham) was added to each well. After a further 5 h culture the cells were harvested onto glass fibre filters with a semi-automated harvester. Incorporation of [^{3}H]TdR into cellular DNA was determined by liquid scintillation counting.

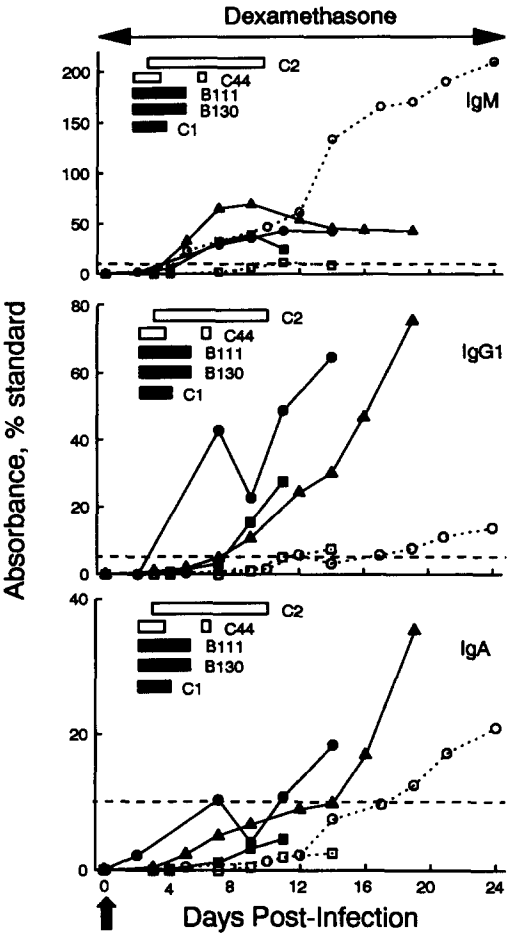

Fig. 1. Serum antibody responses following primary infection with J-160 inoculum (↑) in DX-untreated (Group A, solid lines; B111, ●; B130, ■; C1, ▲) and DX-treated (Group B, dotted lines; C2, ○; C44, □) rotavirus-naive calves. Dexamethasone treatment was started 5 days prior to infection and was by daily intramuscular injection of 0.5 mg kg^{-1} body weight until the end of the experiment (↔). The period of rotavirus excretion in DX-untreated (■) and DX-treated (□) calves is indicated. Horizontal line indicates positive/negative cut off values.

TABLE 2

Faecal IgM and IgA antibody production and rotavirus excretion in calves with and without DX treatment

Group[1]	Calf no.	IgM				IgA				Rotavirus excretion	
		Day 1	Day at peak	Days detectable	Peak level	Day 1	Day at peak	Days detectable	Peak level	Day 1	Last day
A	B111	4[2]	7	6	40.7[3]	11	>12	>2	40	2	5
	B130	6	7	3	26.3		Not detectable[4]			2	5
	C1	7	10	8	28.5	12	13	2	22.8	2	4
B	C2	17	17	2	26.2	20	20	1	39.3	3	10
	C44		Not detectable[5]			12	12	1	22.8	2	6

[1]Group A, rotavirus-infected, DX-untreated; Group B, rotavirus-infected, DX-treated.
[2]Day post-infection.
[3]Absorbance, percent of standard.
[4]Calf B130 only measured up to Day 11 post-infection.
[5]Calf C44 only measured up to Day 14 post-infection.

RESULTS

Effect of DX on serum and faecal antibody responses to rotavirus

Following infection of untreated rotavirus-naive calves (Group A) with the J160 inoculum, antibody responses were demonstrated both in serum and faeces of all three calves tested (Fig. 1, Table 2). Serum and faecal IgM responses were first detectable between Days 5 and 7 after inoculation and reached a maximum between Days 7 and 10; serum IgG1 responses were first detectable between Days 7 and 9; serum IgA responses were first detected on Days 7 and 16 for two of the three calves (the third calf showed no serum IgA response by Day 11 when sampling stopped); faecal IgA was first detectable on Days 11 or 12 in two out of three calves. There was no detectable faecal IgG1 response.

In contrast, DX treatment of rotavirus-naive calves (Group B) suppressed and delayed antibody responses. Serum and faecal IgM responses were only detected in one of the two calves tested. In this calf (C2) the IgM responses were unusual. The serum IgM response did not peak and fall as in the control animals, but continued to rise and, by Day 21 post-infection, reached higher titres than seen in the control calves, while faecal IgM antibody was not detectable until Day 17 and was short lived. Serum IgG1 and IgA also showed different kinetics with a delayed appearance. This was most obvious for IgG1 which was first detectable 14 (C44) or 19 (C2) days post-infection, and was only seen at very low levels, even when followed for up to 21 days post-infec-

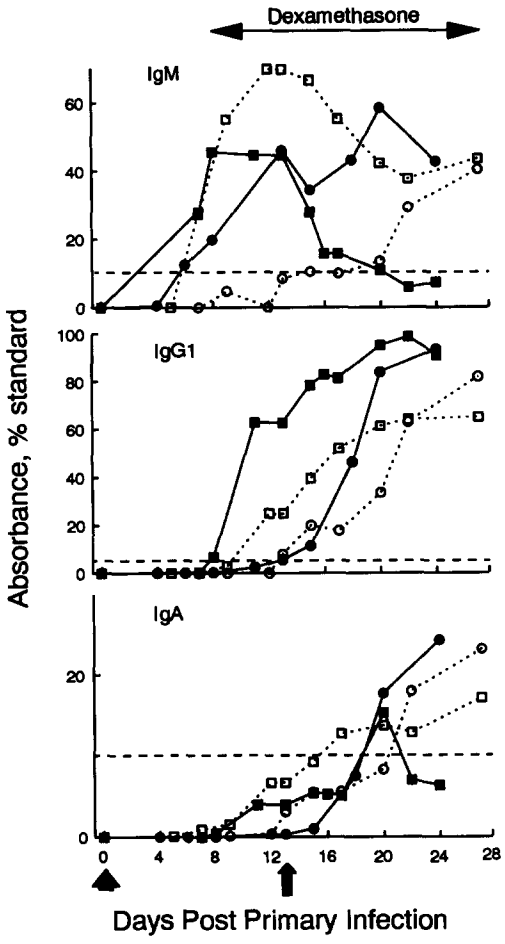

Fig. 2. Serum antibody responses following primary infection with rotavirus C3-160 (↑) and challenge infection with J-160 inoculum (↑) in DX-untreated (solid lines; B110, ●; B150, ■) and DX-treated (dotted lines; B180, ○; C43, □). DX treatment (↔) and other symbols as for Fig. 1.

tion. A serum IgA response, starting at 19 days post-infection, was only seen in calf C2 which was followed beyond Day 14 post-infection. The faecal IgA response of one animal (C2) was delayed and in both calves it was only detectable on one day. The response by calf C44 was similar to that of the untreated calf C1. As in the untreated calves, there were no detectable faecal IgG1 responses.

However, rotavirus-immune calves that had been infected with rotavirus C3-160 8 days before the start of DX treatment showed no discernible suppression of the specific antibody responses either to the primary infection

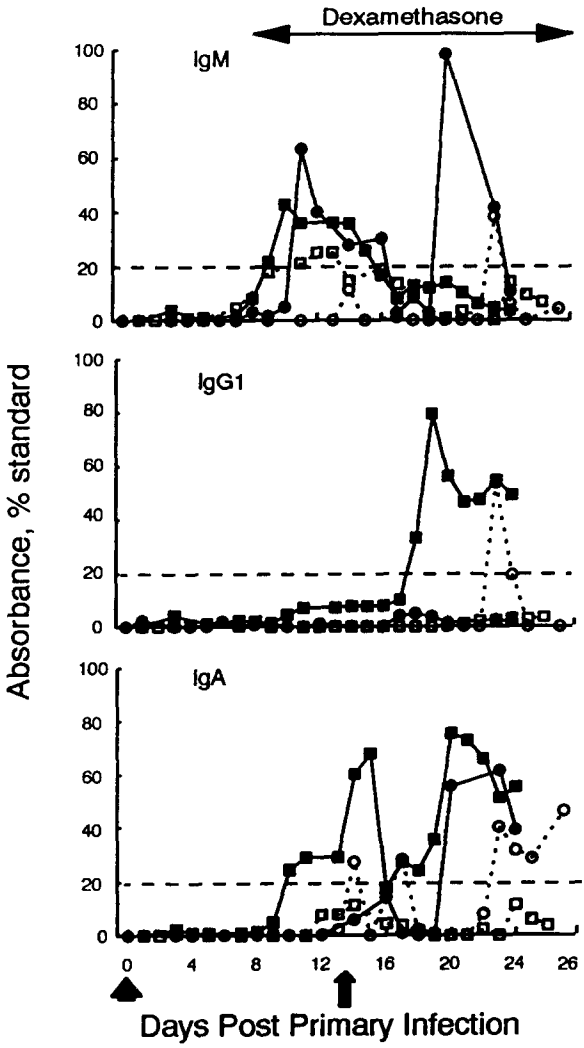

Fig. 3. Faecal antibody responses following primary infection with rotavirus C3-160 and challenge infection with J-160 inoculum in DX-untreated and DX-treated calves. Symbols as for Fig. 2.

or following challenge with the J-160 inoculum on Day 13 (Figs. 2 and 3). The exception was calf B180 which had a suppressed serum IgM response. In contrast to the rotavirus-naive calves, IgG1 antibody was demonstrable in the faeces of two (B150 and B180) of these four calves, 5–10 days after challenge infection (Fig. 3).

TABLE 3

Responsiveness of circulating lymphocytes from rotavirus-immune and rotavirus-naive calves to mitogens before and during treatment with DX

Group[1]	Calf		PHA	Suppression (%)	ConA	Suppression (%)	PWM	Suppression (%)
B	C2	Pre[2]	–[3]		–		–	
		Post[4]	7.1± 2.0 (5)[5]	nd[6]	84.5±47.2 (5)	nd	68.4±42.2 (5)	nd
	C44	Pre	83.0±19.3 (2)		232.8±29.9 (2)		273.8±46.5 (2)	
		Post	7.9± 4.2 (7)	90.5	86.2±30.1 (7)	63.0	37.8±17.4 (7)	86.2
D	B180	Pre	69.3±42.5 (4)		190.8±24.0 (4)		75.0±22.8 (4)	
		Post	11.6± 7.0 (7)	83.3	63.0±17.8 (6)	67.0	35.2±11.7 (7)	53.1
	C43	Pre	234.6±46.9 (3)		205.6±66.2 (3)		266.9±13.0 (3)	
		Post	35.8±21.6 (7)	84.7	61.2±26.1 (7)	70.3	59.4±36.8 (7)	77.1

Peripheral blood lymphocytes were sampled periodically before and during DX treatment and their responsiveness to the mitogens PHA, ConA and PWM determined.

[1]Group B, rotavirus-naive, DX-treated; Group D, rotavirus-immune, DX-treated.

[2]Pre, responses prior to DX treatment.

[3]Cultures lost due to contamination.

[4]Post, responses during DX treatment.

[5]Mean cpm $\times 10^{-3} \pm$ SE (number of observations).

[6]nd, not determined.

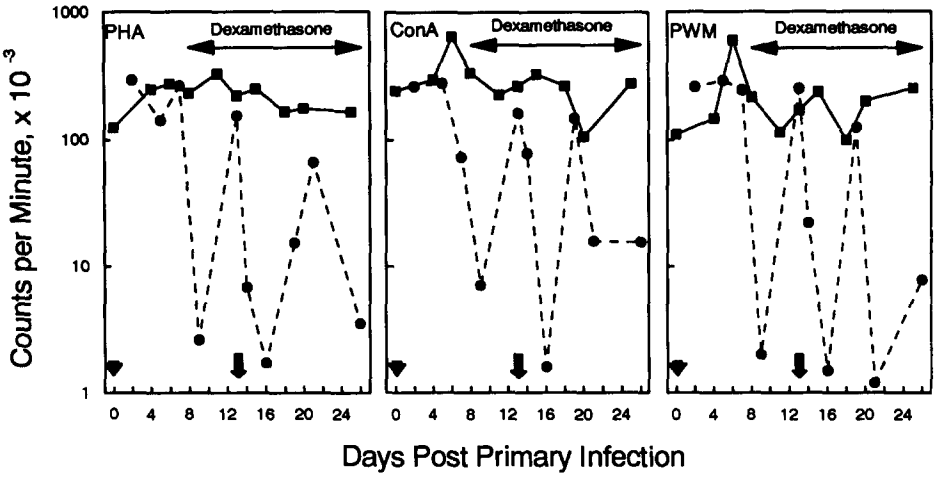

Fig. 4. Proliferative responses to the mitogens PHA, ConA and PWM in DX-untreated (B150, ■—■) and DX-treated (C43, ●- - -●) calves. Calves were infected with rotavirus C3-160 on Day 0 (↑) and challenge infected with J-160 inoculum on Day 13 (↑). Dexamethasone treatment (↔).

Effect of DX on lymphocyte blastogenic responses to mitogens

Circulating peripheral blood lymphocytes, taken during the course of infection, failed to proliferate when cultured in vitro with rotavirus antigens (data not shown). However, the immunosuppressive effects of dexamethasone on cell-mediated immunity was demonstrated with cells cultured with three mitogens. The mean proliferative responses to PHA, ConA and PWM for the 20 day period of DX treatment were reduced by between 53.1 and 90.5% when compared with the responses of the same animals for the period before DX treatment (Table 3). The decrease in mitogen responsiveness was similar for both the rotavirus-immune and rotavirus-naive groups (Groups B and D).

Dexamethasone markedly depressed mitogen responsiveness in all treated calves by 48 h after the commencement of treatment. Although the mean responsiveness to the three mitogens was markedly suppressed during DX treatment, responsiveness re-appeared periodically in all the DX-treated calves. These fluctuations were not seen in control calves. Representative calves from Groups C and D are shown in Fig. 4.

Effect of DX on recovery from primary rotavirus infection

The effect of DX treatment on recovery from primary rotavirus infection was studied in the challenge control calves in Groups A and B. One of the two calves treated with DX (Group B, C2) excreted virus for 8 days rather than

TABLE 4

Effect of DX treatment on recovery from primary rotavirus infection

Group[1]	Calf no.	Number of days with:								Rotavirus excretion	
		Changed demeanour	Anorexia	Abnormal faecal		Dry[3] matter	PCV[4]	Temp[5]	Clinical[6] score	Days positive	Peak[7] titre
				Output[2]	Colour						
A	B111	4	4	5	4	2.5	5	2	26.5	4	5.3
	B130	4	4	4	6	0	0	4	22	4	5.0
	C1	3	1	nr[8]	7	1	0	4	15[9]	3	4.0
B	C2	7	0	15	15	4	0	4	45	8	6.7
	C44	5	1	9	12	2	0	5	32	3	5.4

[1]Group A, rotavirus-infected, DX-untreated; Group B, rotavirus-infected, DX-treated.
[2]> 500 g day^{-1}.
[3]$< 10\%$.
[4]Increased over level seen prior to infection.
[5]> 39.2°C.
[6]Total for all clinical results.
[7]Log_{10} $TCID_{50}$ g^{-1} faeces.
[8]No result – female calf.
[9]Could be higher if faecal output results available.

TABLE 5

Effect of DX treatment on challenge of rotavirus-immune calves

Group[1]	Calf no.	Number of days with								Rotavirus excretion after challenge		
		Changed demeanour	Anorexia	Abnormal faecal		Dry matter[3]	PCV[4]	Temp[5]	Clinical score[6]	Day 1 pi[7]	Days positive	Peak titre[8]
				Output[2]	Colour							
C	B110	0	0	1	2	0	0	2	5	3	6	5.2
	B150	0	2	1	1	0	0	0	4	3	2	4.8
D	B180	0	0	1	0	0	0	1	2	0	0	0[9]
	C43	0	0	2	0	0	0	0	2	0	0	0

[1]Group C, rotavirus-immune, DX-untreated; Group D, rotavirus-immune, DX-treated.
[2]> 500 g day^{-1}.
[3]$< 10\%$.
[4]Increased over level seen prior to infection.
[5]Temperature > 39.2°C.
[6]Total for all clinical results.
[7]Post-infection.
[8]$\log_{10}$ $TCID_{50}$ g^{-1} faeces.
[9]< 1.5 $\log_{10}$ $TCID_{50}$ g^{-1}.

3–4 days as seen in the untreated calves (Group A; Table 4). The peak virus titre in this calf was higher than in the untreated calves but the observed virus titre of $10^{6.7}$ $TCID_{50}$ g^{-1} has been recorded previously with this inoculum in DX-untreated calves (Bridger and Pocock, 1986). The second calf, C44, showed a similar virus excretion pattern to untreated calves. There was no difference between DX-treated and untreated calves in the first day that virus was detected. Both DX-treated calves had a more severe disease than untreated calves: the clinical scores of the DX-treated animals were 32 and 45 compared to 15–26.5 in untreated calves (Table 4). The higher clinical scores were mainly due to increases in the number of days with high faecal output, abnormal faecal colour and low faecal dry matter content.

In the DX-untreated calves, the days on which virus excretion ceased coincided closely with the days on which faecal IgM antibody was first detectable (Table 2). However, in the DX-treated calves, faecal antibody was not detected until Days 12 (IgA) and 17 (IgM) post-infection.

Effect of DX on resistance of rotavirus-immune calves to rotavirus challenge

Dexamethasone treatment did not affect the clinical outcome after challenge. Both DX-treated (Group D) and untreated (Group C) rotavirus-immune calves were resistant to rotavirus disease (Table 5), whereas rotavirus-naive calves (Groups A and B) developed disease (Table 4). However, there were differences in virus excretion between the two rotavirus-immune groups. In the DX-treated immune calves, no virus excretion was detected after challenge (Table 5) but, surprisingly, the untreated immune calves excreted virus at similar levels to that seen in naive calves (compare Tables 4 and 5, Groups A and C) even though they showed no clinical signs.

DISCUSSION

The role of the immune system in recovery from and resistance to rotavirus infection was investigated by suppression of the immune system with DX. Recovery of calves from primary rotavirus infection was affected by DX treatment, although all calves did recover. The duration of clinical signs increased and, in one calf, virus excretion was prolonged. These findings agree with similar studies where DX treatment reduced resistance to primary infection with transmissible gastroenteritis virus (TGEV) in pigs (Shimizu and Shimizu, 1979) and respiratory syncytial virus (RSV; Thomas et al., 1984) and bovine viral diarrhoea virus (BVDV; Clarke et al., 1989) in calves. Termination of viral excretion coincided with the appearance of faecal antibody in DX-untreated calves but not in DX-treated calves. This suggests that antibody-mediated mechanisms are not essential for termination of rotavirus excretion, but may be important in the clinical recovery of naive calves from

rotavirus infection. Although mitogen responses were suppressed they were not completely ablated. However, one must also raise the caveat that DX can affect a variety of immunological and physiological processes other than those measured here, which might have an effect on recovery of the gut from infection and damage. In mice, treatment with cortisone acetate induces the maturation of enterocytes making them resistant to rotavirus infection (Wolf et al., 1981). This did not appear to be occurring in this study as rotavirus-naive DX-treated calves excreted similar levels of rotavirus as the DX-untreated animals. Inhibition by DX of the healing process in the intestine after rotavirus infection may, however, explain the observation that clinical symptoms persisted after virus excretion had ceased.

The clinical outcome after challenge of rotavirus-immune calves was unaffected by DX treatment in contrast to the exacerbation of clinical signs after DX treatment of rotavirus-naive calves. Surprisingly, in the clinically normal, DX-untreated rotavirus-immune calves (Group C), the pattern of virus excretion was similar to that of the clinically-affected DX-untreated rotavirus-naive calves (Group A). However, the uncloned challenge virus was known to be a mixture of rotaviruses (D.H. Pocock, personal communication, 1991) whose individual antigenicities and pathogenicities are not known. Rotaviruses which multiply without causing disease have been described (Bridger and Pocock, 1986; Bridger and Oldham, 1987) and the excreted virus could represent such a virus. Rotavirus was not excreted by the DX-treated calves after challenge however, in contrast to the DX-untreated calves. Crouch et al. (1985) postulated a role for cell-mediated mechanisms in the release of coronavirus particles from infected enterocytes, and a similar mechanism may be involved in rotavirus infections.

Serum and faecal antibody responses in the DX-untreated calves were similar to those reported for rotavirus previously (Van Zaane et al., 1986; Saif, 1987). The suppression of serum antibody responses by DX treatment before antigenic exposure (ie., rotavirus infection) was in agreement with the work of others (Thomas et al., 1984; Pruett, et al., 1987). Our results demonstrate that parenteral DX treatment suppresses mucosal as well as systemic antibody responses. Antibody responses in serum and faeces were not completely abolished but delayed and depressed. The reason for the altered kinetics of antibody production in DX-treated calves is unclear, but it was not due to an inhibitory effect on virus growth by DX.

The timing of GC administration in relation to exposure to antigen affected antibody responses. When DX treatment was started prior to rotavirus infection antibody responses were markedly suppressed, whereas when DX treatment was started 9 days after infection there was no effect on antibody production. This is in agreement with others who have reported that in vivo administration of GC at the same time as exposure to non-replicating antigens can suppress systemic antibody responses in cattle (Pruett et al., 1987),

while suppression of antibody responses to RSV required DX treatment to start prior to infection (Thomas et al., 1984). These studies demonstrate that the immunosuppressive action of DX on antibody production occurs at an early stage in the initiation of the immune response and has no effect on plasma cells.

Differential effects of GC-induced suppression on different immunoglobulin isotypes have been reported (Butler and Rossen, 1973; Roth et al., 1984) but we did not find any. However, Butler and Rossen (1973) looked at pre-existing serum IgM and IgG levels, while Roth et al. (1984) did not measure isotype-specific antibodies but based their suggestion on the finding that responses to *Brucella abortus* were not affected, whereas responses to ferritin and tetanus toxin were.

The present study showed that while immune responses probably speed up recovery from primary rotavirus infection, they are not essential for full recovery. Antibody appeared to have a role in resistance to challenge infection. The existence of non-antibody-mediated immunopathological mechanisms in release of virus from enterocytes was suggested. Further studies are needed to identify the precise mechanisms involved in recovery and resistance to rotavirus infection.

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