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Dexamethasone inhibits virus production and the secretory IgA response in oesophageal–pharyngeal fluid in cattle persistently infected with foot-and-mouth disease virus

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SUMMARY

Cattle persistently infected with foot-and-mouth disease virus were treated with dexamethasone to suppress the immune system in an attempt to influence the level of virus recovery from oesophageal–pharyngeal (probang) samples. Twelve carrier cattle were assigned to one of three groups: control; 0·1 mg/kg dexamethasone; and 0·5 mg/kg dexamethasone. Groups 2 and 3 were injected intramuscularly three times weekly for 3 weeks with dexamethasone between days 33 and 56 post-infection with foot-and-mouth disease virus (FMDV). Cattle in both groups developed a leucocytosis, neutrophilia and lymphopenia. The secretory IgA response to FMDV infection was inhibited following, but not during, dexamethasone treatment between days 70 and 98 post-infection ($P < 0{\cdot}05$). FMDV recovery from probang samples was reduced between days 40 and 64 post-infection ($P < 0{\cdot}05$) during treatment with either 0·1 or 0·5 mg/kg dexamethasone. Following cessation of dosing with dexamethasone virus recovery returned to control levels. These observations suggest dexamethasone inhibits shedding of FMDV in a reversible manner which may be related to its immunosuppressive, anti-inflammatory or physiological actions.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious disease of domesticated and wild cloven-hoofed animals. In young animals the virus can cause a fatal myocarditis but in adults the disease has a high morbidity, low mortality and is characterized by the appearance of vesicles around the mouth, and on the udder and feet. Following recovery from the acute stage, infectious virus can be recovered from the oropharynx for up to 2·5 years in cattle [1]. Animals from which FMD virus (FMDV) is intermittently recovered after 28 days post-infection (PI) are defined as 'carriers'. The epidemiological importance of carrier animals is uncertain [2–4], although they have been implicated in a number of outbreaks following transport and in the transmission from carrier buffalo to their calves [5, 6] and cattle [1, 7, 8]. On the other hand, infection of susceptible animals in contact with carrier cattle has not been demonstrated under experimental conditions. Possible explanations for this include low virus titre in the oropharynx [4], cell-association of persistent virus, presence of neutralizing antibody in secretory fluids [9], dilution and swallowing of secreted virus in saliva and altered virulence/infectivity of carrier virus [10]. In this study immunosuppressive doses of the synthetic glucocorticoid dexamethasone were administered to carrier cattle in an attempt to simulate a stress response and to increase the titre of virus recovered from the oropharynx.

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MATERIALS AND METHODS

Sixteen 6-month-old cross-bred cattle were housed in twos or threes in isolation rooms in the high containment facilities at IAH, Pirbright. They were challenged by the nasopharyngeal route with $\log_{10}$ 5·3 $TCID_{50}$ of strain A24 Cruzeiro FMD virus in phosphate buffered saline (PBS), using a short length of tubing placed in the ventral nasal meatus.

The weight of each animal was estimated using a weighband (Dalton Supplies, Nettlebed, Henley). Animals were examined daily for clinical lesions and rectal temperatures were recorded during the first 10 days PI. Animals from which virus could be recovered by probang sampling (see below), and which were thus defined as carriers on day 28 PI, were assigned randomly into three groups of four animals: controls; 0·1 mg/kg; and 0·5 mg/kg dexamethasone. Dexamethasone (Dexadreson, Intervet UK Ltd, Cambridge) as sodium phosphate (2 mg/ml) was administered three times weekly by two equal volume injections into the gluteal muscles for 3 weeks between days 33 and 56 PI. On the days of dexamethasone administration probang and blood samples were taken before the glucocorticoid injection. In the event of increased viral shedding by immunosuppressed carrier cattle, susceptible animals would be placed in contact in an attempt to demonstrate transmission in a controlled experiment. Animals were killed between days 148 and 154 PI, except two which were killed during the course of the experiment as a result of the animals developing dysentery and becoming recumbent.

Tissues for histology

Tissue samples were collected from dexamethasone-dosed animals SP6 and SP13 and untreated controls at post mortem. The tissues were promptly fixed in 4% paraformaldehyde and later dehydrated and set in paraffin wax. Tissue sections of 4 μm were cut from the paraffin blocks and stained with haematoxylin and eosin. The tissues examined included palatine, pharyngeal and lingual tonsils, retropharyngeal and submandibular lymph nodes and mucosa from the pharynx and soft palate.

Oesophageal–pharyngeal samples (probang samples)

Oesophageal–pharyngeal samples comprising of epithelial scrapings, mucus and saliva were collected twice weekly from day 1 PI until the end of the experiment (day 150) using the probang sampler [3]. Samples were diluted in an equal volume of Eagle's–HEPES medium (pH 7·2) and frozen directly on crushed solid CO_2. Two ml of each sample was transferred to a cryotube for IgA quantification and stored at −70 °C. Primary cultures of bovine thyroid cells (BTY) were used for titrating the FMDV in the probang samples [11]. Four tubes containing tissue culture cells were used per dilution.

FMD virus serotyping by ELISA

To confirm the specificity of the cytopathic effect (CPE) all tissue culture tubes of the same dilution showing CPE were pooled, clarified by centrifugation at 3000 rpm at 4 °C for 10 min and the virus serotyped by ELISA [12].

Indirect IgA ELISA

An indirect ELISA was used to measure FMDV type A24 specific bovine IgA. Ninety-six well flat bottomed Nunc Maxisorb ELISA plates (Nunc, Rosklide) were coated with anti-FMDV type A24 Cruzeiro hyperimmune rabbit serum (50 μl at 1:5000 in carbonate–bicarbonate coating buffer). ELISA plates were incubated overnight at 4 °C. After washing with PBS in a Denley Wellwash 5 automatic platewasher, pretitrated type A 24 FMDV antigen (50 μl at 1:200) was added and the plates incubated on an orbital shaker for 1 h at 37 °C. After washing, a duplicate twofold dilution series of each probang sample was made in blocking buffer (2% casein in PBS) and added to the plates which were incubated for a further 1 h. After washing, monoclonal mouse anti-bovine IgA [13] was added (50 μl at 1:5000 in blocking buffer) and the plates incubated for 1 h. After washing, horseradish peroxidase-conjugated rabbit immunoglobulin anti-mouse immunoglobulin (Dako, Glostrup, Denmark) was added (50 μl at 1:2000 in blocking buffer) and incubated for a further hour. Activated substrate (*O*-phenylenediamine dihydrochloride and H_2O_2) was added (100 μl) and the reaction stopped after 10 min. Optical densities (OD) were read using a Titertek Multiskan spectrophotometer at 492 nm. Antibody titres were expressed in OD at 1/4 probang dilution.

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Statistical analysis

Viral titres in probang samples and FMDV specific secretory IgA results were analysed using one-way analysis of variance.

RESULTS

Virus titres

Of the 16 cattle originally challenged with the A24 Cruzeiro virus 13 became infected and virus was recovered from all on day 28 PI. Virus titres for the first 28 days ranged between $\log_{10}$ 1·25 and $\log_{10}$ 6·5 $TCID_{50}$/ml.

The control group maintained a mean titre of $\log_{10}$ 4–4·5 $TCID_{50}$/ml for the first 28 days, which declined to a mean of $\log_{10}$ 2·5 $TCID_{50}$/ml on day 70 PI and $\log_{10}$ 0·3 $TCID_{50}$/ml by day 148 PI. Virus was isolated from each control animal twice weekly up to day 108 PI. After 108 days virus isolation was intermittent.

The treated groups had similar titres to the control group for the first 28 days. An unexpected and marked decline in probang virus titres soon became apparent at both dose regimes (Fig. 1, Table 1). Following the first injection of dexamethasone there was a decline from $\log_{10}$ 4·0 to $\log_{10}$ 2·5 $TCID_{50}$/ml by day 2 of treatment at 0·1 mg/kg and from $\log_{10}$ 3·75 to $\log_{10}$ 3·0 $TCID_{50}$/ml at 0·5 mg/kg. By day 7 of treatment and following the third dexamethasone injection virus became undetectable with doses of 0·1 mg/kg and declined to $\log_{10}$ 0·38 $TCID_{50}$/ml at 0·5 mg/kg. Virus was isolated from one of eight treated animals on day 7 (day 41 PI), whilst in the control group the mean titre was $\log_{10}$ 3·13 $TCID_{50}$/ml, with virus being isolated from all four animals. Virus was isolated from only two treated animals on one occasion each during the course of treatment between days 33 and 56 PI (day 0–23 treatment). These two isolations were out of a total of 44 probang samples taken from the treated animals. This is in contrast to the isolation of FMDV on every occasion in the control group (24 isolations out of a total of 24 probang samples taken).

Following the cessation of treatment with dexamethasone, virus was recovered from the six remaining animals in the 0·1 and 0·5 mg/kg groups (SP6 and SP13 having been humanely killed on days 48 and 49 PI) between days 56 and 150 PI. All treated animals exhibited a recrudescence in viral excretion in probang samples. In the 0·5 mg/kg group virus was isolated from SP3 and SP7 on day 64 PI (day 8 post-treatment) rising to a maximum on day 72 PI ($\log_{10}$ 4·0 and 3·75 $TCID_{50}$/ml respectively), then declining thereafter. No virus was isolated from SP4 between days 56 and 104 PI, but then titres rose to $\log_{10}$ 3·5 $TCID_{50}$/ml on day 116 PI and later declined to a lower titre of $\log_{10}$ 1·5 $TCID_{50}$/ml at the end of the experiment. The 0·1 mg/kg group showed a similar pattern. Virus shedding by SP17 reached a peak $\log_{10}$ 4·0 $TCID_{50}$/ml on day 80 PI and virus was excreted until the end of the experiment ($\log_{10}$ 1·5 $TCID_{50}$/ml day on 148 PI). SP16 and SP18 exhibited a brief and low level excretion on days 104 and 108. The results are summarized in Figure 1 and Table 1.

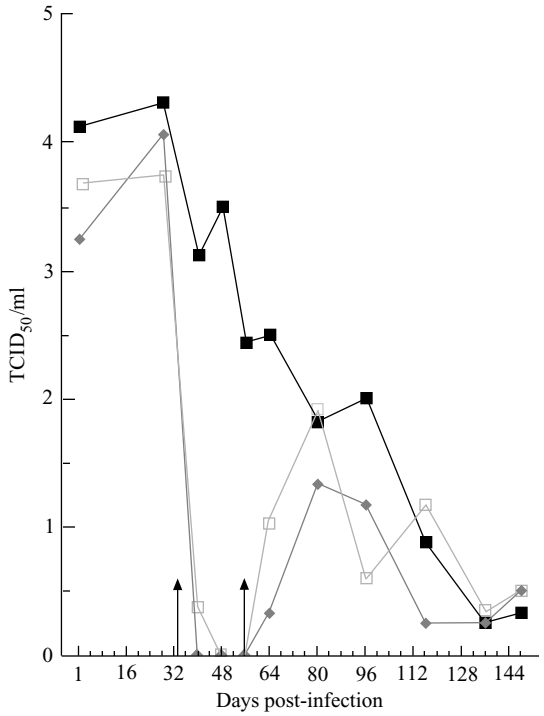

Fig. 1. The mean FMDV oesophageal–pharyngeal (probang) sample titres (see Table 1) against time post-challenge with $\log_{10}$ 5·3 $TCID_{50}$/ml A24 Cruzeiro FMDV. The control group and two treatment groups each contained four animals. Arrows indicate the start (1) and cessation (2) of dexamethasone dosing. ■, Controls; ◆, 0·1 mg/kg dexamethasone; □, 0·5 mg/kg dexamethasone.

A24 FMDV specific secretory IgA

The group of four control animals all showed a significant rise in their mean FMD virus specific IgA by day 42, reaching a peak titre on day 84. The titres remained at this level until day 140 when the mean titre began to decline. The dexamethasone treated groups exhibited a similar pattern to the control

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Table 1. *Mean FMDV titres from oesophageal–pharyngeal (probang) samples*

Day PI	Control	Dexamethasone (0·1 mg/kg)	Dexamethasone (0·5 mg/kg)
1	4·13 (0·48)	3·25 (2·27)	3·69 (1·46)
8	4·5 (0·86)	4·25 (1·76)	4·56 (0·51)
12	3·50 (1·13)	3·50 (0·93)	4·31 (0·55)
20	4·31 (0·51)	3·31 (2·03)	4·56 (0·51)
28	4·32 (0·62)	4·06 (0·68)	3·75 (0·64)
36	3·81 (0·375)	1·19 (0·94)	0·813 (0·62)
40	3·125 (0·77)	0 (0)*	0·375 (0·75)*
44	3·25 (0·79)	0 (0)*	0 (0)*
48	3·5 (0·61)	0 (0)*	0 (0)*
52	2·44 (0·42)	0 (0)*	0 (0)*
56	2·43 (1·07)	0 (0)*	0 (0)*
64	2·5 (0·91)	0·33 (0·57)*	1·08 (1·01)
72	2·56 (0·82)	0·83 (1·44)	2·58 (2·24)
80	1·81 (0·688)	1·33 (2·309)	1·92 (2·00)
88	1·75 (1·00)	0·92 (1·58)	0·58 (1·01)
96	2 (1·06)	1·17 (2·01)	0·58 (1·01)
104	1·5 (0·35)	1·42 (1·28)	1·17 (1·42)
108	1·38 (1·29)	0·92 (0·91)	1 (0·86)
120	0·42 (0·72)	0·33 (0·57)	0 (0)
136	0·25 (0·43)	0·25 (0·43)	0·33 (0·57)
148	0·33 (0·57)	0·5 (0·86)	0·5 (0·86)

Twelve carrier cattle were randomly assigned to one of three groups: control; 0·1 mg/kg dexamethasone and 0·5 mg/kg dexamethasone and injected three times weekly for 3 weeks from day 33 PI.
* $P < 0·05$; ( ), standard deviation.

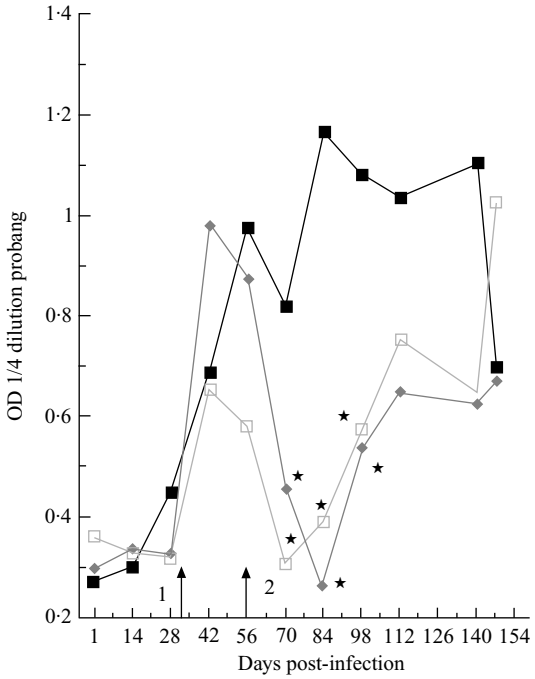

Fig. 2. The mean FMDV specific IgA antibody responses in oesophageal–pharyngeal (probang) samples against time post-infection with $\log_{10}$ 5·3 $TCID_{50}$/ml A24 Cruzeiro FMDV. The control group and two treatment groups each contained four animals. Arrows indicate the start (1) and cessation (2) of dexamethasone dosing. ★ $P < 0·05$. ■, Controls; ◆, 0·1 mg/kg dexamethasone; □, 0·5 mg/kg dexamethasone.

animals for the first 42 days but subsequently differed significantly in their response. The 0·1 mg/kg dexamethasone treated group experienced a decline in their specific IgA titres between days 42 and 84 before rising again to control levels. The 0·5 mg/kg dexamethasone group had a weak initial rise in IgA titre between days 28 and 42 PI and then exhibited a decline in FMD virus specific IgA titres between days 42 and 70 before returning to control group IgA titres on day 112. Both treated groups differed significantly from the control group in titres ($P < 0·05$) between days 70 and 112 PI (Fig. 2).

Histopathology

The effects of dexamethasone on the immune system of the dosed cattle were consistent with its immunosuppressive action. Animals SP6 and SP13 were killed during the course of dexamethasone dosing and there was an unquantified reduction in the amount of lymphoid tissue compared to the untreated controls. The lymphoid follicles of the retropharyngeal and submandibular lymph nodes, the palatine and pharyngeal tonsils and mucosal associated lymphoid tissue (MALT) of animals SP6 and SP13 were reduced in size and number compared to the untreated controls. The MALT was sparsely distributed in the dexamethasone dosed animals and there were very few active follicles with prominent germinal centres.

DISCUSSION

The development of a method for the identification of carrier animals [2] has led to much speculation and experimental investigation of their importance in the epidemiology of foot-and-mouth disease. Field evidence has implicated carriers in a number of outbreaks, although infection of in-contact susceptible animals from carrier cattle has not been shown under experimental conditions. Transmission readily occurs to susceptible animals from clinically affected animals for around 8 days after virus inoculation [14], and sub-clinically infected vaccinated cattle readily transmit up to 7 days PI [15]. After these periods transmission is rare and is particularly infrequent

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from carriers and probably involves as yet undefined predisposing factors.

Glucocorticoids have been shown to have immunosuppressive effects on a range of bovine immunological functions [16, 17] and exacerbate a number of viral, bacterial and protozoal infections [18–21]. In this study we have investigated the effect of using immunosuppressive doses of the synthetic corticosteroid dexamethasone on virus shedding and secretory IgA response in the oropharynx. Surprisingly, we noted a sharp decline in viral titres recovered in probang samples from carrier animals, despite evidence of immunosuppression in haematological, histological and secretory antibody responses.

The effects of glucocorticoids on haematological parameters of the bovine immune system are well documented [16, 22, 23]. The response depends on the type of glucocorticoid, the dose, route of administration and health of the animal. Typically there is a leucocytosis, lymphopenia, neutrophilia and eosinopenia. The effect on monocytes is less clear with some studies demonstrating a monocytosis [22], whilst others show no consistent change [18], possibly explained by the difficulty in distinguishing monocytes from large lymphocytes in the bovine. The marked involution of lymphoid tissue in lymph nodes and aggregations seen in this study would suggest a redistribution of lymphocytes to the bone marrow or other non-peripheral blood compartment, or that they had undergone a glucocorticoid mediated cell-lysis [24].

A previous attempt to induce transmission of FMDV from carrier animals utilizing corticosteroids, parasitic burdens and stress failed [25]. However, the simulated stress of cattle transport resulted in the elimination of the carrier state by the end of the experiment. Artificial stress, induced by the corticosteroid *n*-fluoroprednisolone, did not produce an increase in detectable virus in carrier cattle, nor was there a notable decrease. This may be explained by the potency of the synthetic glucocorticoid administered, the dose, dosing interval and period of administration. In this study much higher doses were injected for a prolonged period (23 days). Interestingly the high titres seen in animals SP17, SP3 and SP7, following dexamethasone treatment is equivalent to titres seen in the early stages of infection and suggests that there may be an increased risk of transmission following, as opposed to during, a stressful episode such as may occur during transport. Despite the presence of secretory antibody in probang samples from these carrier animals the samples proved to be infectious to susceptible cattle when challenged via the nasopharyngeal route (results not shown). The titres of virus recovered between days 56 and 150 PI were frequently above the minimum dose required to infect cattle [26–28].

The effect of dexamethasone on mucosal IgA has been suggested to result from a redistribution of polymeric IgA from mucosal surfaces to serum [29]. The decline of FMDV specific IgA with dexamethasone administration in this study is consistent with this theory. The mechanism by which mucosal IgA production is affected by dexamethasone is unknown. Immunoglobulin synthesis is suppressed in cattle [18, 16] and man [30] treated *in vivo* with glucocorticoids, although stimulation of Ig synthesis has been reported *in vitro* [31]. Alternatively, there may be redistribution of IgA-producing plasma cells away from mucosal surfaces to more central compartments or alterations in the production of secretory component [32]. A final possibility is that the reduction in FMDV specific IgA may result from a reduction in antigen stimulation consistent with the decline in FMDV titres seen in probang samples of the dexamethasone-treated cattle.

Monocytes and macrophages are a key component in antigen presentation, phagocytosis and viral clearance. They are particularly sensitive to the effects of glucocorticoids [33] when compared to other subsets of cells of the immune system with inhibition of all functions in a direct or indirect manner, including chemotaxis, antigen presentation, phagocytosis and cytokine release. Glucocorticoids have been shown to inhibit the release of cytokines and enzymes by macrophages [34], including elastase and collagenase [35], IL-1 [36] and TNF [37] and reduce the expression of FcγR receptors [38]. The inhibition of viral recovery from dexamethasone-treated cattle may be related to the inhibitory effects on cells of the monocyte–macrophage lineage. FMDV infection of dendritic cells derived from porcine skin has been blocked by pre-incubation with African swine fever virus (ASFV) *in vitro* [39] and ASFV interferes with FMDV infection and seroconversion *in vivo* [40]. Negative sense FMDV RNA, as evidence of replication, has been isolated from bovine Langerhans cells [41]. Langerhans cell precursors have been considered as mediators of FMDV transport [42] and these cells readily migrate via lymphatics to lymph nodes and mature into interdigitating cells [40]. Although these authors suggest a mechanism for the early pathogenesis of

FMD, the ability of the virus to replicate in immune cells permits speculation on the possibility of FMDV to persist in cells of the macrophage lineage. Dexamethasone may alter the movement of these cells away from the site of persistence or reduce the turnover of replicating virus within the immune cells. The depletion of lymphoid tissue in the tonsils, lymph nodes and mucosa of the pharynx in the dexamethasone-treated carrier animals may have depleted the immune cells associated with persistence, reduced antigen presentation or altered immune-complex secretion into the oropharynx.

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