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EFFECT OF REPEATED TREATMENT WITH DEXAMETHASONE ON THE RE-EXCRETION PATTERN OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS AND HUMORAL IMMUNE RESPONSE

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ABSTRACT

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Two cattle, free of antibody to infectious bovine rhinotracheitis virus (IBRV) were infected intranasally with IBRV, and developed specific antibody to the virus. Ten weeks later, both animals were given an intravenous course of dexamethasone (DM). Nasal excretion of physical particles of virus, as judged by electron microscopy, occurred in both animals, as early as 24 h after the first DM injection, and high levels of infectious particles appeared several days later. Neutralizing antibody titre to IBRV increased following excretion of virus. Further courses of DM given at 20 and 32 weeks following initial infection were not associated with excretion of physical, 'non-infectious' particles or significant changes in specific antibody titre, although on each occasion one of the two animals excreted low levels of infectious particles.

INTRODUCTION

Infectious bovine rhinotracheitis virus (*Bovid herpesvirus* 1, IBRV) is a member of the herpesvirus group (Armstrong et al., 1961). Several members of this family of viruses are known to become latent in the recovered host, with subsequent intermittent episodes of re-excretion (Honess and Watson, 1977).

Snowdon (1965) reported long-term, intermittent excretions of IBRV, and several authors have recorded that excretion of latent IBRV can be provoked by various stimuli, including corticosteroids (Sheffy and Davies, 1972; Davies and Carmichael, 1973; Darcel and Dorward, 1975). Although the mechanism involved in the initiation of re-excretion is still in dispute, Davies and Carmichael

(1973) have demonstrated that cellular immune responses are involved in controlling the duration of recurrent IBRV episodes, but may not be directly involved in actual reactivation of latent infection. The present report details the effects of DM in relation to excretion of physical and infectious particles of IBRV in immune cattle, and the consequences of repeated stimuli with DM on the same animals.

MATERIALS AND METHODS

Animals. Two 6-months-old cattle, one male, one female, free of antibody to IBRV, were used for initial infection. In addition, one animal with antibody to IBRV was used as a control for delayed hypersensitivity tests.

Viruses. For experimental infection of cattle, strain IBRV/Cu5 was used. This was a local isolate of virus from naturally occurring disease, and had been plaque-purified three times. For neutralization procedures, the international reference strain IBRV/Los Angeles (8th passage in vitro) was used, supplied by Prof. McKercher of Davis University, California. As used in neutralization procedures, the suspension contained a ratio of 1000 physical particles to one infectious particle.

Serum neutralization procedures. Three-fold serum dilutions were reacted with an estimated 100 $TCID_{50}$ of IBRV, and tested in a microtitre system (Davies and Carmichael, 1973), titres being estimated by the Karber method. Indicator cells used were primary or low passage bovine testicular monolayers (BET) grown and maintained in MEM (Flow) supplemented with 10% foetal calf serum (FCS).

Infectivity assays. Ten-fold dilutions of test material were absorbed to monolayers of MDBK cells (Flow) in Petri dishes, and plaques obtained under 1% agarose (Indubiose, IBF) were counted after fixation with formaldehyde and staining with May-Grünwald and Giemsa stains.

Physical particle assays. Suspensions of virus were monitored with the electron microscope, using negative staining, according to the pseudoreplication technique recommended by Smith (1967). Particles were counted on photographs at a magnification of about $\times 15{,}000$.

Delayed hypersensitivity tests. IBRV inactivated antigen was prepared as described by Correa-Giron et al. (1975). Approximately 0.03 ml aliquots were injected intradermally in the neck region, and reactions were evaluated 48 h later by measuring the increase in skin thickness (Aguilar-Setién et al., 1978).

Experimental schedule. At 6 months of age, both animals received 1 ml of a virus suspension containing 8×10^5 plaque-forming units (PFU) into each

nostril. Nasal swabs were collected daily for 20 days and serum samples were taken prior to infection and at weekly intervals for 30 days. Swabs were weighed before and after sampling to obtain an estimate of the volume of nasal excretions tested, and swabs were then eluted in MEM to give an estimated 10^{-1} dilution of the sample. Infectivity assays and electron microscopy examinations were made of this diluted sample after storage at $-20°C$. Occasionally electron microscopy examination was made of undiluted nasal excretions.

At 8½ months of age, animals were intravenously inoculated with six consecutive daily doses of DM (Opticortenol 0.5%, Ciba), at 0.1 mg per kg body weight. Examinations were made daily as above for 16 days, and serum collected weekly for 3 weeks. The procedure was repeated at 11 months and 14 months of age, using double the dosage of DM on the last occasion.

During the third course of DM, blood samples were taken at days 0, 2, 4, 7, 11, 16 and 29 after inoculation, for lymphocyte and total leucocyte counts.

Delayed hypersensitivity tests were also made before and during this third course of DM, antigen being injected at days −3, 0, 4, 7, 16, and 29. A control immune animal was also skin tested at the same time periods.

RESULTS

Initial infection. Daily titration of nasal washings for physical and infectious particles showed that physical particles were excreted for 5 days, and infectious particles for 10 days. The excretion of both types of particles began next day after experimental infection.

The ratio between infectious and physical particles excreted over this period was approximately 1/100. The threshold of sensitivity of electron microscopy for IBRV is about 10^6 physical particles/ml.

First treatment with DM. Excretion of physical and infectious particles during and after the first course of DM is shown in Fig. 1.

The excretion pattern was comparable in both animals. The female excreted high levels of physical particles as early as 24 h after the first injection of DM, whilst infectious particles were not present until the third day of treatment, reaching titres of over 10^6 pfu/ml. The male excreted detectable physical particles 24 h later. Where both physical and infectious particles were excreted, the ratio was fairly constant at 1 infectious to 100 physical particles. Virus excreted by the male on day 6 was serologically characterized as IBRV.

A significant increase in neutralizing antibody titre occurred in both animals by 14 days following the first injection of DM (Fig. 2).

Second treatment with DM. No physical particles were detected in nasal excretions by electron microscopy during or following the second course of DM in either animal, but infectious virus was detected (Fig. 1) on day 6 in the male animal. Neutralizing antibody titres remained stable in both animals over the 20 days following DM treatment (Fig. 2).

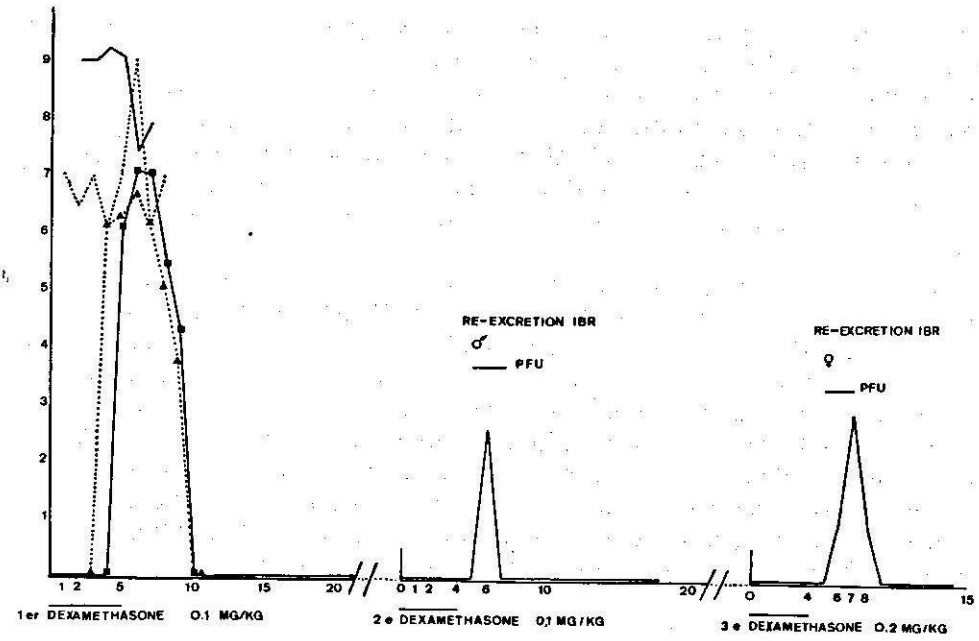

Fig. 1. Re-excretion of viral particles, after repeated treatments with dexamethasone.
———: excretion of physical particles by the male.
■———■: excretion of infectious particles by the male.
. . . . . : excretion of physical particles by the female.
▲. . . . . ▲: excretion of infectious particles by the female.

Third treatment with DM. Using double doses of DM, comparable results to those recorded for the second treatment were obtained, infectious virus alone being detected in the female between days 6 and 8 at a maximum titre of 10^3 pfu/ml (Fig. 1). Neutralizing antibody titres again remained unchanged following DM treatment (Fig. 2).

Other observations. Skin tests performed on the control, immune animal were consistently positive. Skin tests on the treated animals were positive before treatment with DM, but became negative during and following DM treatment, to reappear as positive 17 days following the first DM injection of the third regimen.

White cell counts carried out on both animals during and following the second and third DM treatments, showed that there was an early leucocytosis with a relative lymphopaenia 48 h after the first DM injection, with a subsequent leucopaenia and lymphocytosis, and partial recovery by day 16 post treatment.

DISCUSSION

The excretion of IBRV by experimentally infected animals, and re-excretion,

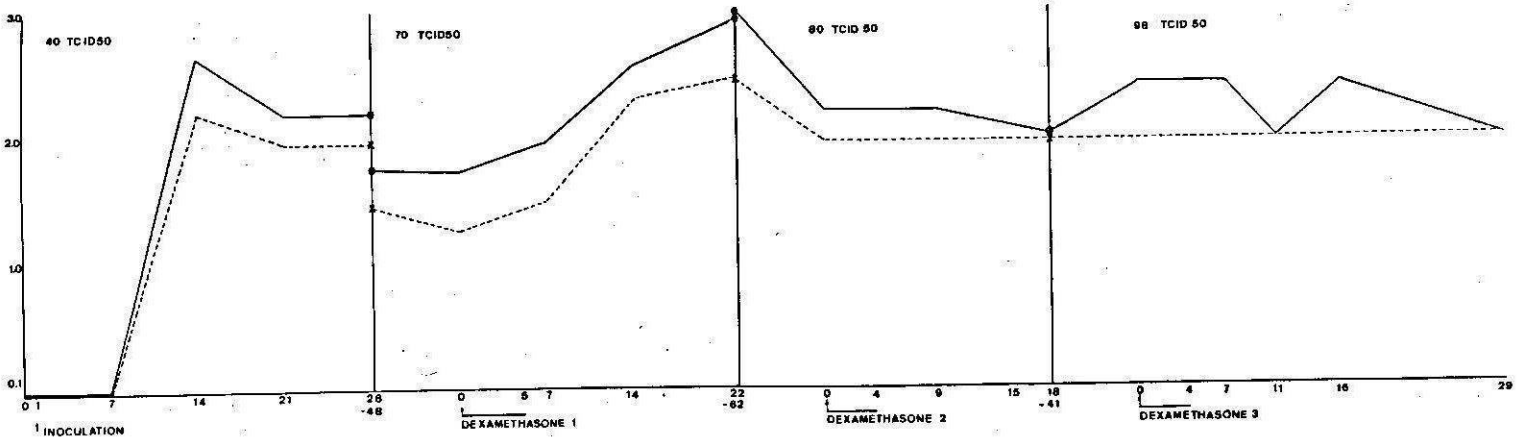

Fig. 2. Neutralizing antibody titres, after repeated treatments with dexamethasone.
- - - - - - - - -: male.
————————: female.
Neutralizing titres are expressed in $\log_{10}$.

following repeated treatments with dexamethasone was studied by monitoring the appearance of physical particles of the virus in nasal washings, and comparing these results with the level of infectious virus present. Twenty-four hours after the first dose of dexamethasone was given, physical particles at levels well above 10^6 per ml were detected by electron microscopy, and these were present for up to 7 days. Infectious virus could not be detected until day 4 in one animal and day 5 in the other, but was still detectable up to day 9 in both animals.

These results suggest that the dexamethasone caused production of non-infectious (probably inactivated) virus particles as a first effect, and that any suppression of immunological mechanisms had only minor effects.

Subsequently, despite demonstration of the pharmacological effects of dexamethasone, as demonstrated by the fall in blood lymphocytes and a negative test for skin hypersensitivity, the second and third treatments with dexamethasone, 20 and 32 weeks respectively after initial infection, produced only a low titre of infectious virus and no physical particles were detected.

These results confirm and extend the observations of Davies and Carmichael (1973) who pointed out that immunosuppression induced by dexamethasone treatment might be of secondary importance, and we think that this substance exerts a direct effect on latently infected cells, causing a recrudescence of herpesviruses.

Our observations may be correlated with those reported by Davies and Duncan (1974) and Narita et al. (1978).

Davies and Duncan (1974) postulated two sites of viral latency following initial exposure to IBRV:

(a) the epithelial tissue in which there was extensive replication initially;

(b) the nervous system providing sensory innervation to that tissue.

A possible explanation for the discrepancy in the excretion of physical and infectious particles following initial infection, and that following the first dexamethasone treatment, with the results obtained following the second and third treatments, might be as follows. After the first exposure to dexamethasone, recrudescence of virus occurs independently at the two sites with a lot of non-infectious virus being produced by nasal epithelial tissue. After the second and third exposure to dexamethasone treatment, recrudescence of virus originated only in the nervous system which could explain the low level observed and the delay in its appearance. Narita et al. (1978) have pointed out that modifications in the nervous system are only present from the third day after injection of dexamethasone.

The alternative explanation could be an increase in the control of reactivation by the immune system, caused by the booster effect of the first reactivation.

Further work is needed to establish whether our observations reflect special conditions of IBRV infection in the bovine animal, and if the use of other strains of this virus will give similar results.

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