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Pathogenesis of equine herpesvirus-1 in specific pathogen-free foals: primary and secondary infections and reactivation

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Summary. Six specific pathogen-free foals shown to be free of equine herpesvirus-1 and 4 (EHV-1 and -4) and lacking in maternally-derived antibodies were used to investigate the pathogenesis of EHV-1 in horses. Following primary intranasal inoculation with EHV-1 all foals showed signs of a mild, self-limiting upper respiratory tract infection. A leucopenia was observed, comprising both a lymphopenia and neutropenia. Virus was isolated from nasal mucus and buffy coat cells over several days during the clinical episode and after the animals became clinically normal. Notwithstanding the mildness of the clinical disease, virus was not eliminated completely and intravenous administration of dexamethasone resulted in reactivation of latent EHV-1 in animals which had received only a single dose of the virus. In a second infection given to four foals, 61 days after the primary inoculation, no clinical signs were observed, haematological changes were minimal and viraemia was absent.

Introduction

Equine herpesvirus type 1 (EHV-1) is a major equine pathogen, responsible for a triad of clinical signs: abortion, neurological and respiratory disease [22]. It is endemic throughout the world, including the U.K. [1]. Notwithstanding its importance, however, the provision of adequate control measures has remained elusive. Vaccines are commercially available but their efficacy is poor [1, 5, 9].

The rational design of more effective immunoprophylaxis has been hindered by the incomplete understanding of EHV-1 pathogenesis and epidemiology. Research into these problems is complicated by several factors. First, the virus is very widespread and, under natural conditions, most equines will have encountered the infection; the first exposure in many cases originating from their dams [1]. Second, the virus, like many other herpesviruses, is able to evade the host immune response and establish a latent or persistent state [7, 11, 14]. Following appropriate stimuli, it may undergo reactivation with the possibility

for recrudescence and/or transmission to susceptible animals [4, 10]. Third, the extent of the interaction between EHV-1 and other equine herpesvirus (EHV-2, 3, and 4), which are also common infections, is unknown [1, 15]: for example, serological measurements are complicated by the cross-reaction between EHV-1 and EHV-4 (e.g., [20]).

Although several studies describe experimental infection of animals seronegative to EHV-1, in many cases, the animals used will have suckled their dams, thus acquiring passive protection, together with the possibility of inapparent maternally-derived infection [3, 8]. The previous use of gnotobiotic or specific pathogen-free (SPF) equines to circumvent this problem has been very limited [12, 21, 24] and many aspects of the disease remain unelucidated. Thus the prevailing situation presents an incomplete and potentially confusing picture about the behaviour of EHV-1 in equines and their response to it.

The present study describes the nature of EHV-1 infections in specific pathogen-free, EHV-free foals, reared as described by Chong et al. [6]. These foals are fully naive for EHV-1 and EHV-4 (and probably EHV-2 and -3), and furthermore lack maternally derived antibody to any of the equine herpesvirus. The effect of intranasal inoculation with EHV-1 strain AB4 is described in fully defined primary and secondary infections. Finally, reactivation of latent virus was achieved by intravenous administration of dexamethasone.

Materials and methods

Animals

Six specific pathogen-free foals, designated F2, F3, F4, F5, F7, and F8, were obtained from Welsh Mountain mares and reared following the procedures developed by Chong et al. [6]. At the start of the experiment the foals were 3 to 4 months of age: F2, F4, and F7 were male; F3, F5, and F8 were female. All foals were shown to be free from EHV-1 and EHV-4 by serological tests and were negative for virus isolation. In addition, they were probably free from the other two equine herpesvirus, EHV-2 and EHV-3, although no specific tests are readily available to detect the presence of these viruses.

Virus strain and tissue culture

EHV-1 strain AB4 was used throughout this study. This strain of EHV-1 was a gift from Dr. N. Edington, Royal Veterinary College, London, U.K., and was originally isolated from a field case which developed neurological signs (paresis). This isolate has also been shown experimentally to cause abortion in pregnant mares (N. Edington, pers. comm.). Monolayers of the rabbit fibroblast cell-line, RK-13, were cultured in Eagle's minimum essential medium (EMEM) supplemented with 10% new-born calf serum. The virus working stock was prepared by infecting RK-13 monolayers in EMEM supplemented with 1% fetal calf serum at low multiplicity of infection. Cell cultures were maintained at 37 °C in a humidified incubator with 5% CO_2. The same working stock (passage 11), with an infectivity titre of 4×10^8 pfu/ml, was used for all foal infections.

Experimental design

The experiment was carried out in 3 phases (Table 1):

1. Primary infection with EHV-1 of four foals (F2, F3, F4, and F5), keeping two foals (F7 and F8) under SPF conditions as uninfected controls.

Table 1. Experimental plan for the infection of six SPF foals with EHV-1 and administration of dexamethasone

	Phase 1 day 0 EHV-1 inf^n	Phase 2 day 61 EHV-1 inf^n	Phase 3 day 103–105 DXM
Foal F 2	primary	secondary	−
Foal F 3	primary	secondary	−
Foal F 4	primary	secondary	+
Foal F 5	primary	secondary	+
Foal F 7	none	primary	+
Foal F 8	none	primary	+

inf^n Intranasal infection with EHV-1, either primary or secondary as indicated

DXM Administration of dexamethasone (+). − No dexamethasone given; foals used as unstimulated controls

None Control foals given uninfected RK cell suspensions to act as controls during the first EHV-1 infection

2. Secondary infection of F 2, F 3, F 4, and F 5 61 days after the primary infection, and at the same time a primary infection of the two previously uninfected control animals (F 7 and F 8).

3. Intravenous administration of dexamethasone 42 to 44 days after the second round of infection to 4 foals. This group comprised both foals (F 7 and F 8) which had received only a primary infection with EHV-1 and two of the foals (F 4 and F 5) which had been inoculated twice. In the third phase of the experiment, two foals (F 2 and F 7) which had been infected previously but which were not given dexamethasone were kept as controls. (A second administration of dexamethasone (same dosage) was given to foals F 4 and F 5, 37 to 39 days after the first treatment; F 2 and F 7 acted as unstimulated controls.)

Until they were infected with virus, all foals were kept under SPF conditions in the positive pressure containment unit (see [6]); the subsequent infections and reactivation experiments were carried out in a separate infection area.

Intranasal (i.n.) inoculation of foals with EHV-1 or control cell suspension

Foals were restrained manually and the inoculum dose administered intranasally (into both nares) in 4 ml of EMEM: half as a liquid suspension via a Pasteur pipette and half as an aerosol. The aerosol was formed using a Sigma spray unit (Sigma, Poole, Dorset, U.K.). After infection, the exact dose of EHV-1 given was determined by titration of a sample of the inoculum to be 7.3×10^7 and 1.9×10^7 pfu in the primary and secondary infection respectively. In the first phase of the experiment, two foals (F 7 and F 8) were given a suspension of sonicated, uninfected RK-13 cells to act as controls.

Clinical assessment

Physical examination (consisting a full clinical examination, including auscultation and examination with ophthalmoscope) of the foals was carried out daily for the first 2 weeks following infections or dexamethasone treatment and intermittently thereafter. ("Depression" (see Table 2) is defined as reduction in normal activity and response to stimuli.)

Table 2. Clinical signs observed in four SPF foals following primary infection with EHV-1

Clinical sign	Day post infection										
	0	1	2	3	4	5	6	7	8	9	10
Loss of appetite	0[a]	0	0	0	0	2	2	2	2	0	0
Depression	0	0	0	0	4	4	4	2	0	0	0
Serous ocular discharge	0	3	4	2	2	1	1	3	0	0	0
Mucopurulent ocular discharge	0	0	0	2	2	0	1	0	0	0	0
Serous nasal discharge	0	1	–[b]	–	–	–	–	–	0	0	0
Mucopurulent nasal discarge	0	0	3	4	4	4	4	3	1	0	0
Submandibular lymph node	0	0	0	4	4	4	4	4	2	2	0
Tracheal noise	0	0	0	0	1	2	1	0	0	0	0
Lower respiratory tract	0	0	0	0	0	0	0	0	0	0	0

[a] The number of SPf foals showing a particular clinical sign
[b] Serous nasal discharge not relevant in foals with mucopurulent discharges

Administration of dexamethasone

In the third phase of the experiment, dexamethasone (Dexadreson, Intervet Laboratories Ltd., Cambridge, U.K.) was administered by intravenous injection at a dose of 2 mg/kg daily for three consecutive days to foals F 4, F 5, F 7, and F 8. As a precaution against bacterial infection the four foals were also given long-acting amoxycillin. Two previously infected foals (F 2 and F 3) were untreated to act as unstimulated controls. Controls consisting of placebo injections were not carried out, but the stress of these would not be greater than that of normal sampling, which included taking intravenous blood samples.

Virus isolation from nasal washings and blood

Mucus was collected from the nasal cavity and nasopharynx of the foals by gentle suction using a foot operated vacuum pump connected to mucus extractors (Unoplast, Hundested, Denmark). Samples were diluted into 1 ml virus isolation medium (EMEM plus antibiotics), mixed thouroughly on a vortex, sonicated at 4 °C and centrifuged at 3,000 rpm for 10 min. Dilutions of the supernatant were then plated onto RK-13 monolayers. Virus was allowed to adsorb for 45 min at 37 °C before the monolayers were overlaid with EMEM plus 1% carboxymethyl cellulose (CMC) and 1% fetal calf serum. Plaques were allowed to develop over the next 2–7 days and when visible the cell sheet was fixed and stained with crystal violet to facilitate plaque enumeration. Any cultures which proved negative for virus after 7 days were re-passaged – the monolayers were scraped off, sonicated at 4 °C, centrifuged at 3,000 rpm for 10 min and aliquots of the supernatant re-plated onto fresh monolayers. In these cases, the original dilutions were re-processed individually and when positive this enabled a quantitative assessment of virus titre in the original sample.

Infectious centre assay

Blood was collected from the jugular vein into EDTA (2 mg/ml) and centrifuged in microfuge tubes. The buffy coat was mixed with distilled water for 1 min to lyse the red blood cells

after which osmotic pressure was restored with 10 × normal concentration of phosphate buffered saline. White blood cells were washed to remove debris, suspended in virus isolation medium and counted by means of an haemocytometer. A known number of cells was then plated onto RK-13 monolayers for 45 min at 37 °C before being overlaid with CMC-containing medium. Plaques were allowed to develop over 2 weeks, during which the cells were fed with additional EMEM supplemented with 10% neonatal calf serum. When plaques were visible they were fixed and stained using crystal violet and enumerated. Any plates which did not develop plaques were re-passaged as detailed above.

Fluorescent antibody test (FAT) and virus neutralisation test

Samples of white blood cells were spun down onto glass slides (cytospins), air-dried and fixed in ice-cold acetone. These samples were used for indirect immunofluorescence staining of EHV-1 antigen containing cells as described in Awan et al. [2]. Briefly, the cells were reacted with polyvalent anti-EHV-1 hyperimmune serum raised in rabbits and then sheep anti-rabbit Ig FITC conjugate.

The virus neutralization test was carried out using rabbit polyvalent anti-EHV-1 hyperimmune serum (neutralising titre 1/512) as described in Awan et al. [2].

Restriction endonuclease analysis

DNA was extracted from virus stocks using standard procedures (e.g., [17]). Digestion with EcoRI and BamHI was carried out using 2 units enzyme/μg of DNA. The digested samples were then run on 0.7% agarose gels and the bands of DNA stained with ethidium bromide and visualized under ultraviolet light.

Results

Primary infection of specific pathogen-free EHV-free foals

Four SPF foals were inoculated intranasally with EHV-1 strain AB4. Two foals were given a suspension of sonicated, uninfected RK-13 cells to act as controls.

Clinical signs

The four foals infected with AB4 became abnormal on the day following infection. Over the next 7 to 9 days they displayed a variety of clinical signs: ocular and nasal discharges, depression and inappetence, and swollen submandibular lymph nodes (Table 2). Clinically, the severity of the disease was at its height by days 4 to 5, and thereafter the animals began to recover. By day 7 clinical signs were minimal and they had disappeared by day 9 with the exception of one foal (F 3) which developed bilateral ocular complications 3 weeks following infection (see Discussion). Corneal lesions were not observed. The overall picture was one of a mild, transient upper respiratory tract infection which was self-limiting. No lower respiratory tract involvement was clinically apparent.

The two control animals showed no adverse reaction to intranasal inoculation of an uninfected cell suspension.

Rectal temperature

Rectal temperature was taken daily for the first 10 days p.i. and intermittently thereafter. Following infection, rectal temperature began to increase within a day, peaking biphasically on days 2 and 6 before returning to normal values by days 7–8 (Fig. 1). Temperature transiently declined below normal values on days 10–12.

Haematology

Total white blood cell (wbc) counts and differentials were monitored daily for 10 days p.i. and then at decreasing frequency. A biphasic leucopenia was observed, with minimum values obtained on day 2 and days 6–7 (Fig. 2). The low cell counts comprised both a lymphopenia and neutropenia (Fig. 2). Lymphocyte numbers recovered and increased to a peak on days 10–13 before declining to pre-infection levels by day 20, and thereafter showed a slow rise.

Virus isolation

Virus recovery from nasal mucus and buffy coat samples was attempted at regular intervals for 5 weeks p.i. Nasal excretion of virus was observed over the first 8–9 days p.i. in all foals, and for 14 days in F 5 (Fig. 3). Again the data were suggestive of a biphasic behaviour: thus virus was undetectable in some animals on days 5–7 p.i. before reappearing on days 8–9. Samples taken after 14 days were negative for all foals. In addition, all samples taken on days 4–7 p.i. were negative for the presence of virus on first passage in RK cells and required re-passaging before virus became apparent.

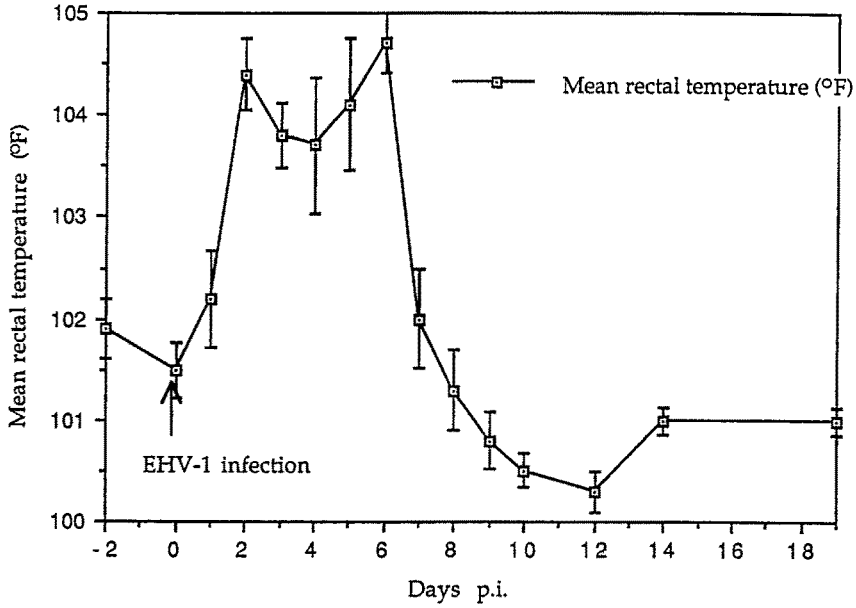

Fig. 1. The effect of a primary intranasal infection with EHV-1 on rectal temperature of SPF foals. Each point represents the mean ± S.E. of four foals

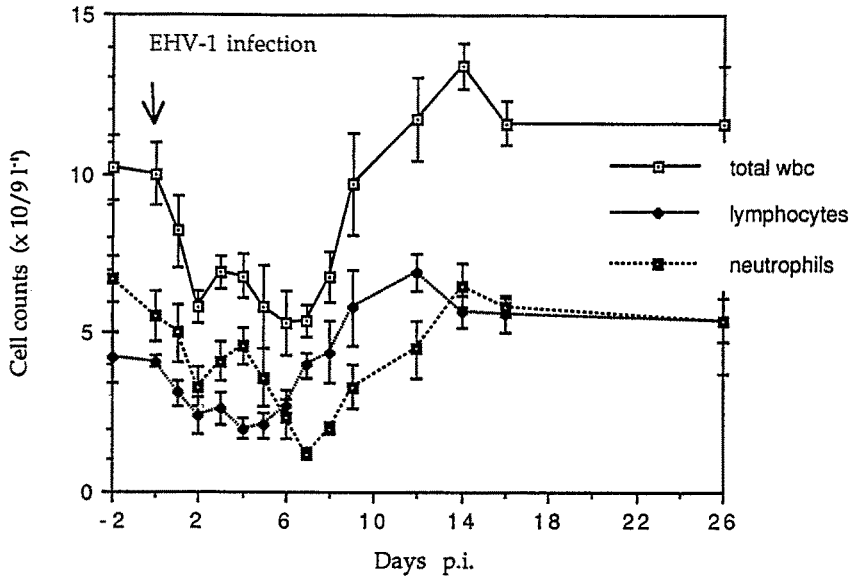

Fig. 2. Changes in total wbc, lymphocyte and neutrophil counts ($\times 10^9 l^{-1}$) of four SPF foals following a primary intranasal infection with EHV-1. Each point represents the mean ± S.E. of four foals

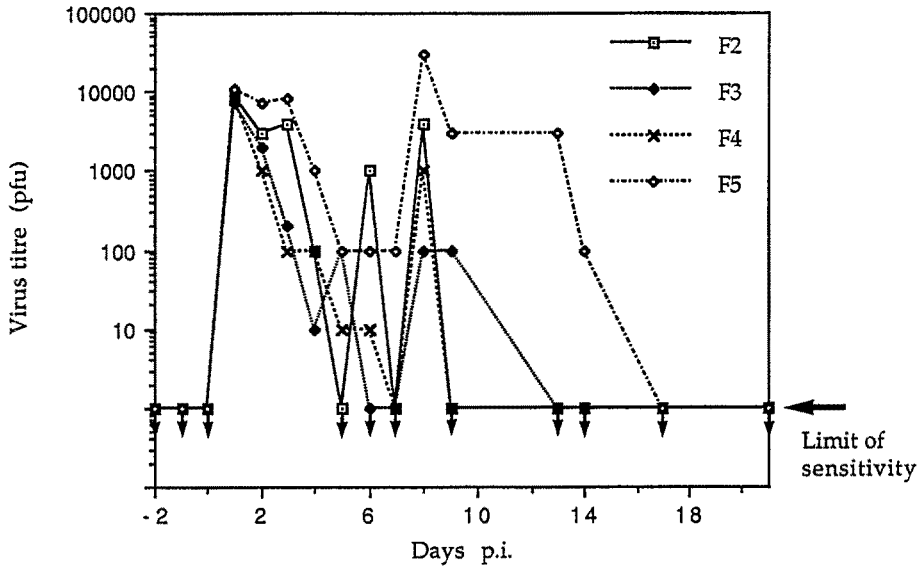

Fig. 3. Individual virus titres (pfu/sample) in nasal samples taken from four SPF foals following a primary intranasal infection with EHV-1

Viraemia was undetectable by the infectious centre assay for the first 5 days p.i. On days 6–9 p.i. all foals were positive for the presence of virus in the blood, with F 5 again continuing to yield virus on days 14 and 16 (Table 3). It was notable that virus plaques from infectious centres appeared only slowly

Table 3. Infectious centres (given as infectious centres/10^6 cells) grown from buffy coat cells taken from four SPF foals on various days following an intranasal infection with EHV-1

Day p.i.	Foal number			
	F 2	F 3	F 4	F 5
1–5	0	0	0	0
6	5	15	370	140
7	136	20	3	1
8	32	0	8	4
9	16	17	1	82
12	0	0	0	0
14	0	0	0	2
16	0	0	0	+
>19	0	0	0	0

0 Samples negative for presence of virus even after passaging
\+ Sample found to be positive for virus after blind passage

(cf. those from nasal mucus) and required 7–10 days of incubation before they could be readily enumerated. Isolates were confirmed as EHV-1 by the virus neutralisation test.

Second infection with AB 4

In the second phase of the experiment, both groups of foals were inoculated intranasally with EHV-1, strain AB 4, i.e., a primary infection for the two foals previously given uninfected RK-13 cells (F 7 and F 8), and a second infection for foals F 2–5, 61 days after the first inoculation. An identical virus stock was used at the same dose with the same method of administration.

Primary infection of foals F 7 and F 8

The effect of intranasal inoculation of foals F 7 and F 8 was very similar to that observed in the first round of infection. Clinical signs lasted for about 7 days and rectal temperature was again elevated biphasically over this period (Fig. 4). Virus was recovered from nasal mucus at high titres for 7 or 11 days p.i. in foals F 7 and F 8 respectively (Fig. 5). A leucopenia occurred during the first week of infection (data not shown). In contrast to the infection described above, however, on this occasion no viraemia was detected by means of infectious centre assay. That viraemia did occur, despite these negative results, was suggested by the presence of virus antigen-containing cells seen by immunofluorescence on day 5 p.i. in F 8, and days 7 and 9 in both F 7 and F 8.

Second infection of foals F 2 to F 5

On re-infection with the same strain of EHV-1, a very different picture was seen. No clinical signs were apparent, including no change in rectal temperature

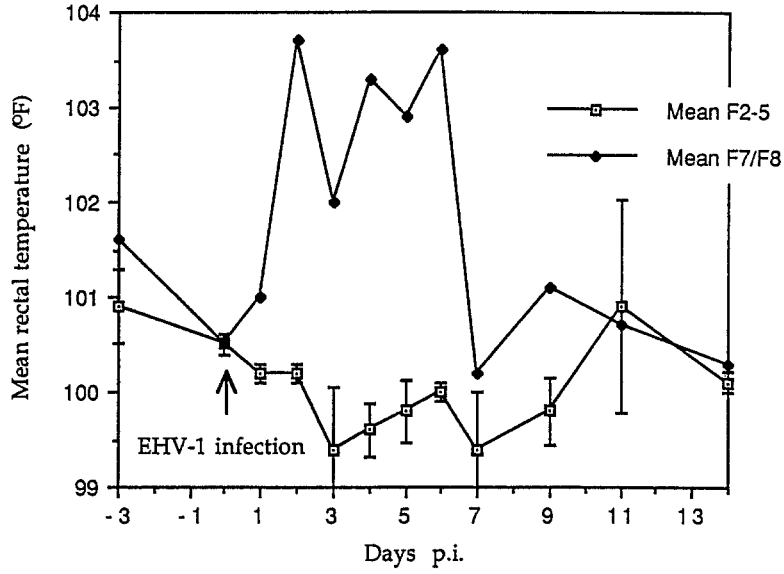

Fig. 4. The effect of a secondary intranasal infection with EHV-1 on mean rectal temperature (± S.E.) of four SPF foals (F 2–5). Mean rectal temperature from two foals (F 7 and F 8) receiving a primary infection is included as control

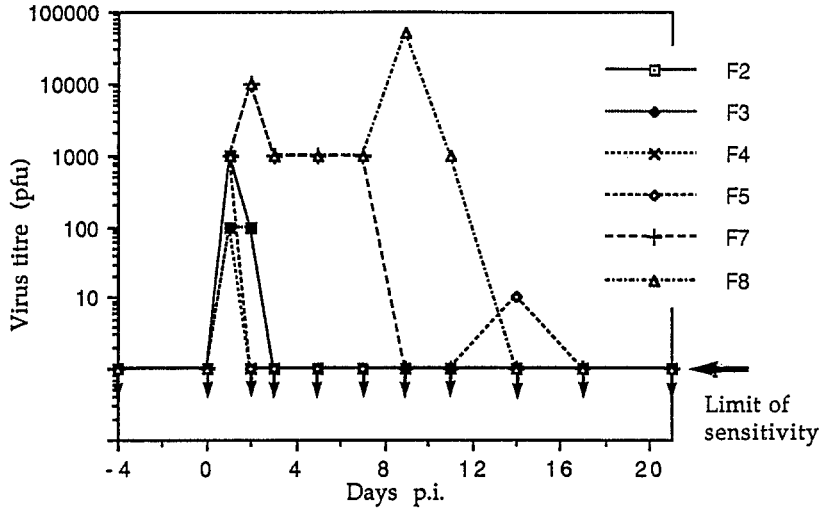

Fig. 5. Individual virus titres (pfu/sample) in nasal samples of six SPF foals following either a primary intranasal infection with EHV-1 (*F7* and *F8*) or a secondary infection 61 days after the first exposure (*F2, F3, F4,* and *F5*)

(Fig. 4). Similarly, haematological parameters (total wbc count, lymphocytes and neutrophils) were unchanged (Fig. 6). Viral recovery was attempted over a period of 21 days after the second infection. Nasal excretion of virus, however, was seen only for the first 2 days p.i. (with the exception of F 5 from which virus was also isolated on day 14 p.i.) and even these samples required re-

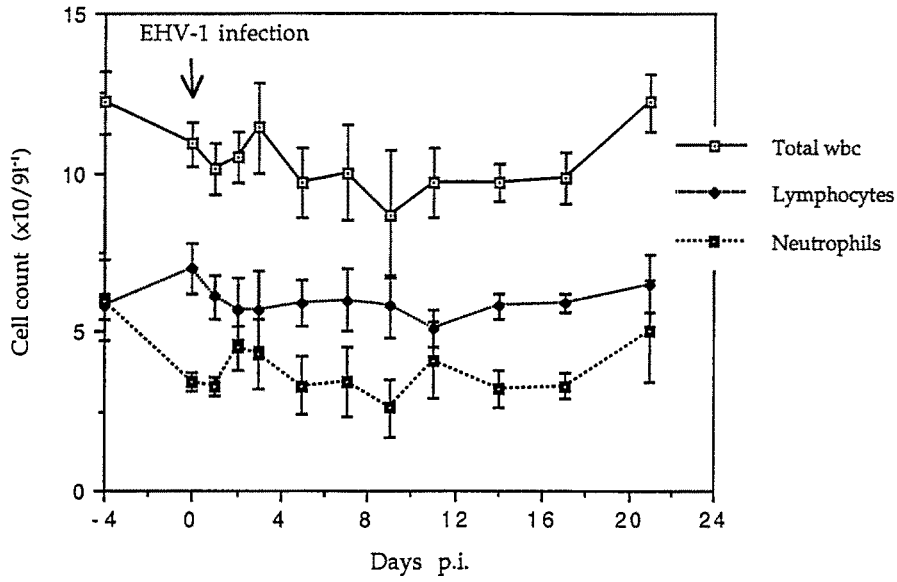

Fig. 6. Changes in total wbc, lymphocyte and neutrophil counts ($\times 10^9 l^{-1}$) following a secondary intranasal infection with EHV-1, 61 days after the primary exposure. Each point represents the mean ± S.E. of four foals

passaging before CPE were visible (Fig. 5). Viraemia was undetectable by either infectious centre assay or immunofluorescence in the foals given a second infection.

Administration of dexamethasone

In the third phase of the experiment an attempt was made to re-isolate virus from nasal mucus and blood following intravenous administration of dexamethasone. Four foals (F 4, F 5, F 7, and F 8) received dexamethasone; F 4 and F 5 had been given two inoculations with AB 4 15 and 6 weeks previously, whilst F 7 and F 8 had received a single inoculation 6 weeks previously (see above). Two infected foals (F 2 and F 3) were kept as unstimulated controls.

Blood and nasal samples taken from the 6 foals before administration of dexamethasone were all shown to be negative for virus or virus antigen-containing cells. Virus was isolated from nasal mucus of all 4 stimulated foals (Table 4) over a period of 5 to 12 days after the first dexamethasone injection. In all cases the samples required re-passaging before CPE were visible. Two foals (F 4 and F 7) yielded virus on each of several days; F 5 and F 6 were positive on single days only.

The re-isolated virus was shown to be EHV-1 by FAT and this was confirmed by restriction endonuclease analysis. The pattern of fragment migration was identical for re-isolate and inoculum samples, consistent with their identity.

Infectious centre assays, taken on the same days as nasal mucus samples, were always negative. FAT, however, on cytospins of buffy coat showed the

Table 4. Virus isolation from nasal mucus of four foals after intravenous administration of dexamethasone (2 mg/kg daily for three days)

Days following start of dexamethasone i.v.	Foal number			
	F 4	F 5	F 7	F 8
1	−	−	−	−
3	−	−	−	−
5	−	−	+	−
8	+	−	+	−
10	+	−	+	+
12	+	+	−	−
15	−	−	−	−
17	−	−	−	−

+ Virus isolated
− Virus undetectable

presence of virus antigen-bearing cells on day 8 for F 4 and F 5, day 14 for F 7 and day 10 for F 5, F 7, and F 8 (Fig. 7).

No virus was detected in the two control (i.e., infected but unstimulated) foals and all their cytospin samples were negative for immunofluorescence to EHV-1.

A second attempt at reactivation was carried out 37 days later in two foals, F 4 and F 5, following the same protocol. One of these, F 4, again showed nasal excretion of virus 5 days after commencement of dexamethasone treatment (data not shown).

Discussion

The most important feature of the present work was the use of specific pathogen-free (EHV-free) animals which, at the start of the experiment, were free, with reasonable certainty, from all four equine herpesviruses (see Note added in proof). In addition, since the foals were separated from their dams at birth and, therefore, had no chance to suckle, no maternally-derived antibody was present. The majority of previous observations on EHV-1 infections have been carried out on experimental animals whose EHV status is unknown. Even if seronegative when infected, suckled foals would have acquired passive maternal antibodies together with the possibility of inapparent infection with EHV-1 or EHV-4.

The principal findings were as follows. First, the administration of a large intranasal dose of a virus strain known to be associated with paresis and abortion produced, in these naive foals, only mild clinical signs. No apparent lower respiratory tract involvement or neurological signs were observed with the exception that one foal (F 3) developed bilateral blindness (Slater et al., manuscript in prep.). Second, the animals were solidly immune to a second inoculum of the same strain of virus 61 days after the primary dose. Finally, a suitable

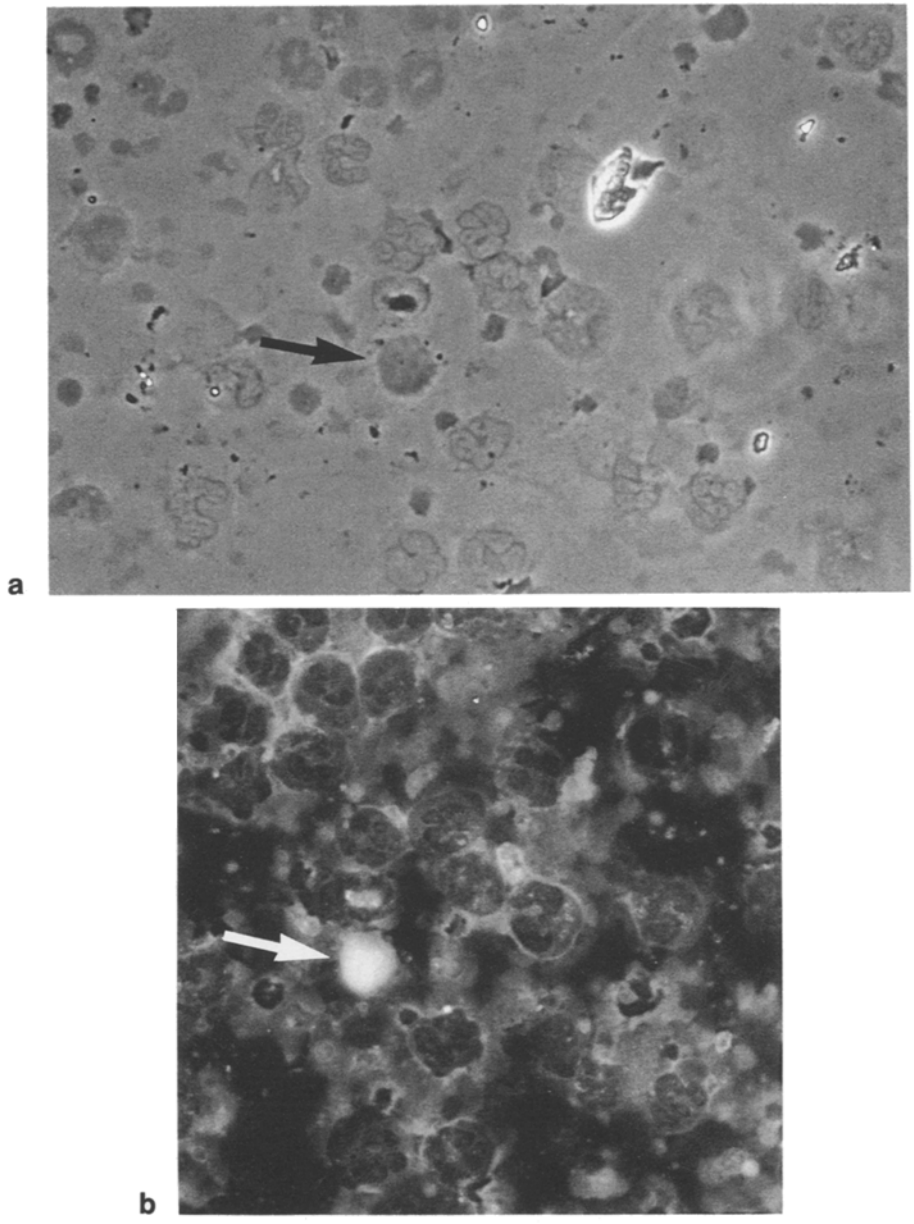

Fig. 7. Fluorescent antibody test using hyperimmune rabbit anti-EHV 1 antiserum for the presence of EHV-1 antigen on buffy coat cells from foal F 7 9 days after the first injection of dexamethasone (2 mg/kg i.v. daily for three consecutive days): **a** cells viewed under phase contrast, **b** cells viewed under ultraviolet light. Both mononuclear and polymorphonuclear cells are visible. The arrows indicate a cell positive for EHV-1 antigen. Controls using buffy coat samples pre-infection or using pre-immune rabbit serum were all negative (not shown). × 470

stimulus (intravenous dexamethasone) caused recurrent shedding of virus into nasal mucus 42 days after the secondary inoculum (or 103 days after the primary inoculum).

These findings are very similar to the results obtained from infection of seronegative (but non-SPF) animals receiving intranasal infections of EHV-1 [1, 13]. Likewise, the lack of clinical signs during the second infection resembles that seen in repeat infection of initially seronegative foals or primed adult horses. It has been reported that such infections have been accompanied, in some cases, by viraemia [3], despite the absence of clinical signs, though this was not the case in the present study. The relatively short period (61 days) between infections may account for this difference.

The present results demonstrate that immunologically naive foals are able to respond briskly to a very pathogenic strain of EHV-1. A large component of their initial response presumably comes from non-specific immune defence mechanisms. Interferon levels in nasal mucus in SPF foals infected with EHV-1 rise rapidly following infection (Chong et al., unpubl. obs.) and NK cell activity is also high. The foals did seroconvert (Gibson et al., manuscript in prep.) but the appearance of humoral antibodies would seem to be too slow to account readily for the rapid remission of clinical signs, thus limiting the immunity provided by antibody dependent cell-mediated cytotoxity and complement dependent effects as suggested previously [19]. In addition, the present results imply that the severe disease sometimes produced by EHV-1 must involve other host or environmental factors, e.g., stress, mixed infections or superinfections [18].

The present observations cannot account fully for the reasons behind changes in white blood cell numbers. Leukopenia is a frequent finding in experimental and natural infections of non-SPF animals although neutropenia or lymphopenia alone are observed in some cases [12, 24]. It is tempting to hypothesise that the decrease in lymphocyte numbers is caused by specific virally mediated destruction which may be involved in some form of immune suppression. Sequestration of cells in areas of inflammation, e.g., respiratory tract, is also a possibility. The changes in lymphocyte populations were analysed further by the use a panel of monoclonal antibodies directed to equine lymphocyte surface markers in conjunction with fluorescence-activated cell sorting [13 a].

On re-infection with EHV-1 from the same virus working stock and at a similar dose the foals were apparently immune in that no clinical signs were observed and virus could be obtained from nasal mucus for 2 days only. One foal (F 5) did show nasal excretion of virus on day 14 p.i. also, but this may have been due to a spontaneous reactivation of latent virus. Viraemia was also undetectable by infectious centre assay or immunofluorescene. The control (naive) foals, however, showed a response similar to that seen in the first round of infections with the exception that virus was not isolated from cultures of buffy coat. That viraemia did occur was suggested by fluorescent antibody test for EHV-1 antigen on cytospins of buffy coat samples. This illustrates a major

problem with EHV-1. Viraemia is thought to be central to the pathogenesis of the virus leading to the characteristic sequelae of abortion or neurological signs. It is, however, difficult to detect reliably by infectious centre assay. The use of DNA hybridisation tests, for example the polymerase chain reaction technique, is being developed in this laboratory and elsewhere [16] and such approaches may provide a more sensitive test.

A similar problem was encountered with some of the nasal samples. In some cases, the samples did not reveal the presence of virus until they were re-passaged and this was especially true in those samples taken after administration of dexamethasone. The reason for this phenomenon may include the presence of interferon and/or neutralizing antibody or that the virus was present in only low titres. It is also possible that, even in the acute phase of infection, virus is actually being reactivated in vitro from cells which contain virus in a latent or non-productive state. The inability to isolate virus after the acute phase of infection was over, in the absence of stimulation with dexamethasone, however, indicates that virus must exist in qualitatively different states during acute and latent phases. In contrast to experimental infection of non-SPF animals [24], neutralization tests showed that only EHV-1 was present.

In the final phase of the experiment, dexamethasone was administered intravenously in an attempt to reactivate latent virus. The results show that reactivation did indeed occur in all four foals stimulated and the virus proven to be EHV-1. Although experimental reactivation of EHV-1 has been reported previously it has not been described in animals whose EHV-1 status is fully known. Two of the four foals (F 7 and F 8) had received only a single intranasal infection of the virus; the other two (F 4 and F 5) had received two such inocula. Thus a single uncomplicated intranasal infection with EHV-1 was sufficient to establish a latent state from which virus could be reactivated with the potential of transmission to susceptible animals. That reactivation was reproducible was shown by the ability to isolate virus from one out of two foals given a second treatment with dexamethasone, giving a total of five reactivations from six attempts.

Experimental reactivation of latent EHV-1 has been shown previously [10]. In this case, a combination of dexamethasone (1 mg/kg i.v.) and prednisolone (2 mg/kg i.m.) was administered and produced findings similar to those obtained above with dexamethasone alone, except that comparison of inoculum and re-isolated virus demonstrated an inconsistency in the endonuclease restriction pattern. This suggests that the reactivated virus may in fact have been a different strain from that inoculated experimentally, thus illustrating the difficulties of working with non-SPF animals.

The present results, therefore, confirm and extend work on the pathogenesis of EHV-1 performed in non-SPF equines. It shows that the use of SPF animals for studying many aspects of the disease, including latency, is feasible. Furthermore, SPF foals allow the interaction of the several equine herpesviruses to be examined in detail. The serological responses and changes in lymphocyte populations will be the subject of a further communication.

Note added in proof. A fluorescent antibody test on foal sera showed that the animals were free from antibodies against EHV-2 at birth and also during both EHV-1 infection periods. Several foals, however, seroconverted to EHV-2 after administration of dexamethasone (Dr. N. Edington, pers. comm.).

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References

1. Allen GP, Bryans JT (1986) Molecular epizootiology, pathogenesis and prophylaxis of equine herpesvirus-1 infections. Prog Vet Microbiol Immun 2: 78–144
2. Awan AR, Chong YC, Field HJ (1990) The pathogenesis of equine herpesvirus type-1 in the mouse: a new model for studying host responses to infection. J Gen Virol 71: 1131–1140
3. Bryans JT (1969) On immunity to disease caused by equine herpesvirus-1. J Am Vet Med Ass 155: 294–300
4. Burrows R, Goodridge D (1984) Studies of persistent and latent equine herpes-1 and equine herpesvirus-3 infections in the Pirbright pony herd. In: Wittman G (ed) Latent herpesvirus infections in veterinary medicine. Nijhoff, Boston, pp 307–319
5. Burrows R, Goodridge D, Denyer MS (1984) Trials of an inactivated equid herpesvirus-1 vaccine: challenge with a subtype-1 virus. Vet Rec 114: 369–374
6. Chong YC, Duffus WPH, Field HJ, Gray DA, Awan AR, O'Brien MA, Lunn DP (1991) The raising of equine colostrum-deprived foals: maintenance and assessment of specific pathogen (EHV-1/4) free status. Equine Vet J 23: 111–115
7. Doll ER, Bryans JT (1963) Epizootiology of equine viral rhinopneumonitis. J Am Vet Med Ass 142: 31–37
8. Doll ER, Wallace ME, Richards MG (1954) Thermal, haematological and serological responses of weanling horses following inoculation with equine abortion virus: its similarity to equine influenza. Cornell Vet 44: 181–190
9. Dutta SK, Shipley WD (1975) Immunity and the level of neutralization antibodies in foals and mares vaccinated with a modified live-virus rhinopneumonitis vaccine. Am J Vet Res 36: 445–448
10. Edington N, Bridges CG, Huckle A (1985) Experimental reactivation of equid herpesvirus-1 (EHV-1) following the administration of corticosteroids. Equine Vet J 17: 369–372
11. Erasmus BJ (1966) The activation of herpesvirus infections of the respiratory tract in horses by immunisation against horse sickness. In: Proceedings of the 1st international conference on equine infectious diseases, pp 117–121
12. Fitzpatrick DR, Studdert MJ (1984) Immunologic relationships between equine herpesvirus type-1 (equine abortion virus) and type-4 (equine rhinopneumonitis virus). Am J Vet Res 45: 1947–1952
13. Gleeson LJ, Coggins L (1980) Response of pregnant mares to equine herpesvirus-1 (EHV-1). Cornell Vet 70: 391–400

13a. Lunn DP, Holmes MA, Gibson JS, Field HJ, Kydd JH, Duffus WPD (1991) Haematological changes and equine lymphocyte subpopulation kinetics during primary

infection and attempted re-infection of specific pathogen free foals with EHV-1. Equine Vet J [Suppl 12]: 35–40
14. Mumford JA (1991) The epidemiology of equid herpesvirus abortion: a tantalising mystery. Equine Vet J 23: 77–78
15. O'Callaghan DJ, Allen GP, Randall CC (1978) Structure and replication of equine herpesviruses. In: Proceedings of the IVth international conference on equine infectious diseases. Veterinary Publications, Princeton, pp 1–31
16. Onions D (1991) Equine herpesvirus: new approaches to an old problem. Equine Vet J 23: 6–7
17. Owen LJ, Field HJ (1988) Genomic localization and sequence analysis of the putative bovine herpesvirus-1 DNA polymerase gene. Arch Virol 98: 27–38
18. Prickett ME (1969) The pathology of disease caused by equine herpesvirus-1. In: Proceedings of the 2nd international conference on equine infectious diseases, pp 24–33
19. Stokes A, Wardley RC (1988) ADCC and complement-dependent lysis as immune mechanisms against EHV-1 infection in the horse. Res Vet Sci 44: 295–302
20. Thomson GR, Mumford JA, Campbell J, Griffiths L, Clapham P (1976) Serological detection of equid herpesvirus-1 infections of the respiratory tract. Equine Vet J 8: 58–65
21. Thomson GR, Mumford JA, Plowright W (1978) Immunological responses of conventional and gnotobiotic foals to infectious and inactivated antigens of equine herpesvirus-1. In: Proceedings of the IVth international conference on equine infectious diseases. Veterinary Publications, Princeton, pp 103–114
22. Timoney JF, Gillespie JH, Scott FW, Barlough JE (1988) Hagan and Bruner's microbiology and infectious diseases of domestic animals. Comstock, Cornell
23. Turner AJ, Studdert MJ, Peterson JE (1970) Persistence of equine herpesviruses in experimentally infected horses and the experimental induction of abortion. Aust Vet J 46: 90–98
24. Wilks CR, Coggins L (1978) An assessment of lymphocyte transformation and cytotoxicity following infection with equine herpesvirus type-1. In: Proceedings of the IVth international conference on equine infectious diseases. Veterinary Publications, Princeton, pp 93–101

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