Experimental transplacental transmission of porcine cytomegalovirus

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SUMMARY

Six serologically negative sows were infected by intranasal instillation of porcine cytomegalovirus (PCMV) between 31 and 85 days of pregnancy. Four sows showed an afebrile anorexia and lethargy 14–25 days after infection and all 6 developed significant increases in indirect immunofluorescent (IIF) antibody titres within 35 days. Virus was recovered from nasal and/or cervical swabs from 2 sows during life and from lung macrophage cultures after death.

At term the sows were killed and their fetuses harvested by caesarean section. The number of mummified and stillborn fetuses increased from 4/63 in 6 previous litters to 18/60 in the 6 present litters. Nine of 43 fetuses born alive were reared in isolators for up to 6 weeks but the majority were killed for examination on the day of birth. Virus was isolated from 16 piglets from 4 of the 6 litters examined; it was isolated most frequently from lungs and liver but also from spleen, kidney, brain and nasal mucosa. Unsuckled day-old pigs had insignificant IIF titres, irrespective of whether they were excreting virus or not. The 5 congenital excretors which were reared all died within 7 days but no deaths occurred among their 4 litter-mates. Post-natal infection of 2 of these piglets reared in contact with congenitally infected pigs was suggested by the recovery of virus from nasal swabs 17 and 27 days after birth and the subsequent rise in IIF titre to 1/256 by day 42.

INTRODUCTION

Surveys have indicated that porcine cytomegalovirus (PCMV) infection is widespread in commercial herds of pigs in Britain (Plowright, Edington & Watt, 1976). It has also been clearly demonstrated that, quite apart from its role in the pathogenesis of rhinitis, PCMV infection in the newborn can result in fatal generalized disease, with the virus showing a predilection for reticulo-endothelial cells (Corner, Mitchell, Julian & Meads, 1964; Edington, Plowright & Watt, 1976*a*).

Rac (1961), in Australia, was the first to identify transplacental infection when he observed cytomegaly with characteristic intranuclear inclusions in the nasal mucosa of a day-old pig. In Canada, L'Ecuyer, Corner & Randall (1972) confirmed

congenital transmission of the virus in 8 of 11 litters, having previously suspected transplacental contamination in a minimal disease herd by the introduction of caesarean-derived piglets.

With the present trend in pig husbandry towards the formation of very large breeding herds of minimal disease status, it is important that the potential dangers of transplacental PCMV infection should be more clearly defined. This paper records our preliminary investigations in this field.

MATERIALS AND METHODS

Experimental animals

The breeding sows came from a caesarean-derived, barrier-maintained herd set up at the Central Veterinary Laboratory, Weybridge in 1963; it has never shown any evidence of PCMV infection.

Virus inoculum

The B6 strain of virus (Plowright *et al.* 1976) was given to each sow as an intranasal instillation of 2 ml of tissue culture fluid containing $10^{4.8}$ TCD₅₀.

Isolation of virus

Portions of nasal mucosa, lung, kidney and liver were taken from piglets and sows killed at term, and from piglets which subsequently died or were killed at 6 weeks of age; placental tissue and cervical mucosa were also taken. Finally, direct cultures were made from lung macrophages of all experimental animals (Edington *et al.* 1976*a, b*). Tissues and fluids expressed from nasal or cervical swabs in maintenance medium were stored at -70°C until used for virus recovery in pig lung macrophage (PLM) preparations (Plowright *et al.* 1976).

Leucocyte fractions from peripheral blood were treated as previously described (Plowright *et al.* 1976).

Serology

Indirect immunofluorescent (IIF) tests for IgG antibody were performed as previously described (Plowright *et al.* 1976). Sera collected weekly from nine piglets reared in isolators were examined for the presence of specific IgM antibody, using a suitable dilution of an anti-pig IgM fraction prepared in rabbits and purified by affinity chromatography on cyanogen bromide gels; this was followed by the application of a commercial ovine anti-rabbit conjugate.

Fetal development

The age at death of those fetuses which had failed to survive to term was assessed radiographically (Wrathall, Bailey & Hebert, 1974) and by measurements of fetal length and body weight.

Histopathology

Pieces of the tissues sampled for virus isolation were fixed for routine histopathological examination, as also were blocks of brain, spleen, submandibular and bronchial lymph nodes, gonad, mandibular salivary gland and Harderian gland.

Experimental procedure

Pregnancy was confirmed 30 days after mating by the vaginal biopsy technique of Done & Heard (1968) and the sows isolated from the herd. All were initially serologically negative. Four sows (nos. 1-4) were inoculated intranasally with PCMV at between 57 and 85 days of gestation, i.e. when the conceptus is considered to have become immunocompetent (Bourne, Curtis, Johnson & Collings, 1974) and a further 2 (nos. 5 and 6) were infected at 31 and 36 days respectively (Table 1). Nasal and cervical swabs, buffy coat and serum samples were collected at least once a week for examination as described above. Rectal temperatures were recorded daily.

Intramuscular injections of 200 mg of progesterone on days 110 and 112 of gestation were followed by slaughter of the sows at term and the harvesting of their litters by caesarean section. The piglets from sows 1 and 2 were all killed for virological, histological and radiographic examination shortly after delivery. From 3 subsequent litters (sows 3, 4 and 6; Table 1) some neonates were sacrificed immediately but groups of 2 or 3 of them were reared in gnotobiotic isolators (Trexler, 1971). Nasal swabs were obtained from these animals twice and serum once a week; buffy coat samples were collected twice a week from two pigs in each litter. Surviving animals were killed at the end of 6 weeks and tissues taken as described above.

RESULTS

Sows

Between days 14 and 25 after exposure, four of the six sows became anorexic and lethargic for periods of 3-9 days (Fig. 1) but no significant fluctuations in temperature were recorded. Infection in all six sows was confirmed by the development of IIF antibody, the mean titre rising steadily between 1-5 weeks and thereafter more quickly to reach a plateau of 1/194 at 7 weeks after exposure (Fig. 1). Virus (PCMV) was isolated from nasal swabs between days 21 and 30, and from cervical swabs between days 30 and 35; lung macrophage cultures revealed virus at slaughter in sows 3 and 4.

Although fetuses from four of the six sows yielded virus (Table 1), PCMV was never isolated or detected cytologically in placental tissue, nor was there histological evidence of primary placental damage in fetuses which had recently died.

Fetal deaths

Following infection with PCMV the 6 sows produced litters comprising 60 pigs, of which 18 were mummified, whereas 6 previous litters from 5 of these sows produced a total of 63 piglets including only 3 mummified fetuses and 1 stillborn

Fig. 1. IIF titres in relation to virus isolation and clinical signs in six sows infected with PCMV. △, Isolation of virus from cervix. ▲, Isolation of virus from nasal cavity. □, Anorexia.

Fig. 2. Ages at which fetuses died following PCMV infection of six sows at various stages of pregnancy.

animal (Table 1). Radiographic examination of the 18 dead fetuses indicated that 13 of these had died 4–6 weeks after inoculation of the sow and that this time relation was independent of the stage of pregnancy (Fig. 2). Typical mummified fetuses were shrunk and dehydrated and covered by tenacious brownish-black membranes. Fetuses which had died more recently were swollen and autolysed with a greyish discoloration.

Autolysis precluded virus isolation in 16 cases and histological examination was often equally fruitless. However, cytomegaly and intranuclear inclusions were seen in the liver and/or lung of three dead fetuses, and both virus recovery and histological confirmation were positive in the two dead piglets delivered 80 days after inoculation of the sow.

Table 1. *Fetal deaths in the litters of 6 sows before and after PCMV infection*

Sow	Previous breeding history		Litters after PCMV infection			
	No. of fetuses	No. dead	No. of fetuses	No. dead	No. infected	Day of pregnancy infected
1	12	0	14	3	7	80
2	10	0	12	2	0	57
3	11	0	12	3	3	85
4	8, 11	1, 3	11	4	4	71
5	11	0	5	5	2	31
6	.	.	6	1	0	36
Total	63	4	60	18	16	

Table 2. *Nasal excretion of PCMV and the distribution of virus at necropsy in 9 piglets from sows 3 and 4*

Isolator	Pig no.	Day of death	Period of nasal excretion (days after delivery)	Virus isolation at Necropsy			
				Nasal mucosa	Lung	Kidney	Liver
I	A	3	.	—	+	+	+
	B	42	.	—	—	—	—
II	C	6	0-6	+	+	+	+
	D	42	27-38	—	+	+	—
III	E	6	0-6	+	+	+	+
	F	42	17-42	+	+	+	+
IV	G	1	.	+	+	+	+
	H	7	0-7	+	+	+	+
	I	42	.	—	—	—	—

Post-natal observations on reared piglets

A total of 14 pigs were reared from 3 sows. All 5 pigs from sow no. 6, infected at 36 days of pregnancy, were negative for PCMV. The results of virus isolation from 9 pigs in the remaining 2 litters (from sows nos. 3 and 4), each of which included both infected and virus-free animals, are summarized in Table 2. At least one pig in each isolator unit died within 7 days of delivery and in each case PCMV was detected in lung, liver and kidney tissues (5/9). All 4 survivors remained clinically normal and 2 (B and I) remained virologically and serologically negative, though the other 2 (D and F) excreted PCMV in nasal secretions on days 27-38 and 17-42 respectively. In these 2 animals IIF titres began to rise 10 days after virus was isolated and attained 1/256 and 1/512 by the time of necropsy at 42 days; PCMV was subsequently isolated from their tissues (Table 2).

All of 23 neonatal sera from these litters were negative for IIF antibodies, using either IgG or IgM antiglobulin preparations. Fetuses that had died close to term, probably within 48 h, and newborns that died within 7 days of birth did

not show any macroscopic lesions of diagnostic value. Histologically, intranuclear inclusion-bodies were frequently observed in the liver and/or lung (10 cases), followed by the spleen and kidney (5 and 6 cases respectively) and then by nasal mucosa and brain (4 cases). Sinusoidal or capillary endothelial cells were most commonly affected but inclusions were also present in hepatocytes and lung macrophages.

DISCUSSION

The cultural and/or cytological detection of virus in 16 piglets taken surgically from 4 sows confirmed that transplacental infection with PCMV can occur at any stage of pregnancy between 31 and 85 days.

The pattern of PCMV infection in the 6 sows, with excretion of virus from nasal mucosa or cervix between 3 and 5 weeks and peak antibody titres about 7 weeks after infection, closely resembled that in gnotobiotic pigs infected at the age of 3 weeks, which had shown a viraemia 14–16 days after infection (Edington *et al.* 1976*b*). The occurrence of 12 fetal deaths 28–42 days after the infection of sows suggests that the sows in the present experiments probably experienced a viraemia at about this time with PCMV crossing the placental barrier and replicating in the fetus within 4–5 weeks of the sows being exposed to infection. It could be that the depression and anorexia in sows 14–29 days after exposure was associated with a viraemia, although no virus recoveries were made from their leucocytes in these experiments.

Infection of sow 5 at 31 days of pregnancy resulted in a small litter of 5 pigs, all of which were mummified. Early fetal death is also sometimes observed following other virus infections during pregnancy, e.g. swine fever virus (Dunne, 1970), the SMEDI group of picornaviruses (Dunne *et al.* 1969) and the herpes virus of Aujeszky's disease (for review see Baskerville, McFerran & Dow, 1973). As with most other virus infections (Wrathall, 1975) PCMV infection casualties were also located randomly in the uterus but in our experiments most of the deaths (13/18) occurred between 28 and 40 days after maternal exposure, 6 being at 28 days. However, the fact that 4 of the sows were given virus at 57–85 days of pregnancy meant that in the case of 12 of the mummified fetuses, fetal life could only have lasted a maximum of 30–58 days after infection. Of the 5 mummified fetuses from sow 5, 2 had died at 40 days, 1 at 56 days and the remaining 2 at 78 and 79 days respectively after maternal exposure. As virus was isolated from both these last piglets, the question arises whether virus crossed the placenta on a number of occasions, whether it had replicated less quickly in some fetuses or whether these casualties resulted from spread by contact from one fetus to others in the uterus.

Of the 16 fetuses demonstrably infected with PCMV, 10 from 3 litters were alive at term; 5 of these were placed in isolation and all 5 died within 7 days. Of 4 litter-mates, which were apparently free of PCMV at birth and housed in contact with some virus excretors, 2 contracted infection and developed high titres of IIF antibody. A high mortality rate in congenitally infected infants is not a feature of CMV infection in man but in this species virus-neutralizing antibody is normally

transferred to the fetus via the placenta (Reynolds *et al.* 1973). Evidence for the regular transfer of colostral antibody has already been obtained (Plowright *et al.* 1976) and it could be that colostral antibody normally gives some protection to newborn piglets infected transplacentally with PCMV.

No congenitally infected piglets had PCMV antibody of either IgG or IgM class at birth, which suggests that either they were immune-tolerant or that the degree of antigenic stimulation was inadequate to produce antibody at term, as found by Jamrichova, Sokol & Sevcik (1971) for 88-day fetuses inoculated with low doses of an attenuated strain of pseudorabies virus. Immune tolerance has been postulated by Levinsohn *et al.* (1969) for a small number of congenitally infected children who subsequently shed virus persistently. However, in man immune tolerance would seem to be exceptional, as all of the 38 cases described by Stagno *et al.* (1975) had high titres of IIF antibody which were maintained for up to 5 years. Binns (1967) has shown that the porcine fetus becomes capable of producing antibodies to lymphoid cell antigens between 60 and 80 days of gestation. Bourne *et al.* (1974) have also demonstrated that antibodies were formed *in utero* against porcine parvovirus given at the 58th but not on the 55th day of gestation, whilst Bachmann, Sheffy & Vaughan (1975) using direct inoculation of the same virus into fetuses, found that an antibody response was first elicited between 55 and 72 days.

Both Cameron-Stephen (1961) and Corner *et al.* (1964) reported deaths in litters from sows affected with rhinitis and perinatal infection, suggesting PCMV was the cause. L'Ecuyer *et al.* (1972) detected PCMV in 28 out of 83 piglets comprising 8 of the litters born to 11 sows which were infected intranasally at various stages of pregnancy; they described stunting, pneumonia, rhinitis and death in affected piglets, from 9 days after birth. This proportion of congenitally infected piglets correlates closely with our observations (16/60). They also recorded an increase in intra-uterine deaths but did not make a clear differentiation between congenital and post-natal infection (Corner, Randall & L'Ecuyer - personal communication).

In women several workers have observed an increase in the cervical excretion of human CMV during the last trimester of pregnancy (Numazaki *et al.* 1970; Montgomery, Youngblood & Medearis, 1972; Stagno *et al.* 1975). This occurred in the presence of circulating maternal antibody and the consequences for the infants involved were not known. Cervical excretion of PCMV has not previously been recorded in sows and its frequency and significance in breeding herds needs to be assessed. Further work is necessary to determine whether the recrudescence of PCMV excretion occurs in 'carrier' sows and whether boars may be responsible for transmission.

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