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PLAQUE MORPHOLOGY AND PATHOGENICITY FOR NEWBORN MICE OF SWINE VESICULAR DISEASE VIRUS. I. WILD STRAINS AND THEIR CLONES

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Summary. — The population of wild swine vesicular disease virus (SVDV) strains was found non-homogeneous as manifested by varying plaque size and different pathogenicity of the clones obtained. The clones derived from large plaques (5—9 mm)—dominating among wild strains—were more virulent for newborn mice than those obtained from smaller plaques (1—2 mm). To evaluate the pathogenicity of wild SVDV strains the dose index was calculated; the clones were compared by dose index and theoretical pathogenicity index, respectively.

Key words: *swine vesicular disease virus*; *large- and small-plaque clones*; *pathogenicity evaluation*; *newborn mice*

Introduction

Recently genetic investigations were performed on various isolates of the swine vesicular disease virus (SVDV) by the plaque method (Brown and Wawrzkiewicz, 1977; Niemiałtowski, 1977; Dilovski, 1978; Malicki *et al.*, 1979).

Brown and Wawrzkiewicz (1977) demonstrated a greater similarity between some Coxsackievirus B5 (C B5) and SVDV clones than among different clones of the same strain. Malicki *et al.* (1979) proved in pigs and baby mice the higher pathogenicity of clones obtained from large (6—10 mm) as compared to small (0.5—1 mm) plaques of wild SVDV and C B5 strains. This confirmed the observations on the great similarity in certain biological properties of definite SVDV and C B5 clones.

The present study was undertaken to characterize the wild SVDV strains and their clones on the basis of their plaque morphology and of their pathogenicity to newhorn mice. The characteristics of SVDV temperature-dependent mutants derived from strains and their clones is given in the next paper (Niemiałtowski, 1983).

Materials and Methods

Viruses. Three wild SVDV strains were examined; SVDV/1, SVDV/2 and SVDV/3 isolated from diseased swine. Standard SVDV strain Poland 1/73 was used in serum neutralization tests (Brooksby, 1973).

Following viruses were used as control strains in the immunofluorescence (IF) test: Coxsackie B5 (C B5) and poliomyelitis (LSc-2ab) viruses, foot-and-mouth disease virus, serotype C (FMDV), vaccinia virus (VV) and equid/alpha/herpesvirus 1 (RAC-H).

Standard immune antiserum. Immune antiserum was obtained from piglets experimentally infected with Poland 1/73 strain of SVDV. The antiserum was then applied for neutralization test and for preparation of the fluorescent reagent.

Cell cultures. Viruses SVDV, VV and RAC-H were grown in a culture of IBRS-2 cells (pig kidney cell line). Cultures on Petri dishes 5 cm in diameter were used for SVDV cloning. The dishes were kept at 37 °C in ASSA-T 303 GF incubator at humidified 5% CO_2 atmosphere. C B5 and LSc-2ab viruses were grown in CMK cells (*Cercopithecus aethiops* kidney epithelial cells), FMDV was grown in primary calf kidney cells.

Plaque technique. Small-plaque and large-plaque clones were isolated from wild SVDV strains by three-fold cloning according to the two-layer agar technique (Dulecco and Vogt, 1955) modified in our laboratory (Niemialtowski, 1977: Malicki *et al.*, 1979).

Identification of the SVDV strains and clones. Wild SVDV strains were identified by serum neutralization (SN) in its alpha modification (decreasing virus doses against constant serum dose) and by direct immunofluorescence (IF) test. The clones were identified by direct IF and by SN in plaque inhibition test.

Determination od the influence of chemical compounds on wild SVDV strains. Cation thermostabilization in 1 mol/l magnesium chloride and 1 mol/l calcium chloride was tested at 50 °C for 1 hr and sensitivity of SVDV to 10% chloroform, 20% ethyl ether and 0.25% trypsin was assayed by routine procedures (Larski, 1977).

Biological test on 1-day-old newborn mice. LD_{50} of the wild SVDV strains and their clones was determined on baby "Swiss" mice. For each virus dilution one litter of 8 newborn mice was used. Various dilutions of the viral material were injected to the baby mice subcutaneously in a 0.05 ml volume. Two controls were applied in each experiment; (a) two litters in which the newborn mice received Eagle's medium 1959 (MEM) in a 0.05 ml volume and (b) two intact, untreated litters. The mice were kept under observation for 20 days. Virus that killed the sucklings was identified by direct IF in the impression smears of suckling mouse brains.

Comparison of the pathogenicity of SVDV strains and clones. For this purpose the dose index and the theoretical pathogenicity index were calculated. The dose index (DI) of the wild SVDV strains and their clones were calculated according to the formula:

$$DI = \frac{TCID_{50}}{LD_{50}}$$

The theoretical pathogenicity index (TPI) for clones of wild SVDV strains was calculated according to the formula;

$$TPI = \frac{DI_s}{DI_l}$$

where DI_s = DI for the small-plaque clone and DI_l = DI for the large-plaque clone.

Statistical evaluation. For comparison of the arithmetic means of plaque diameters the variance analysis in one-way classification and Duncan's test (Elandt, 1964) were applied.

Results

Identification of SVDV strains and their clones

In SN slight differences were noted in the neutralization index between the tested wild strains and the standard SVDV strain. They amounted to 0.1—0.6 log indicating that the investigated strains are homologous to the standard strain. Standard immune serum neutralized the virus clones obtained from wild strains. All SVDV strains and their clones reacted positively

Table 1. Pathogenicity of wild SVDV strains for newborn mice

Strain	Virus titre*		Dose index
	$TCID_{50}$/ml	LD_{50}/ml	
SVDV/1	5.5*	4.8*	0.7
SVDV/2	5.8	5.3	0.5
SVDV/3	5.7	4.7	1.0
Control I**	—	0.0	—
Control II***	—	0.0	—

* In $\log_{10}$ values ** Uninfected mice *** Mice receiving Eagle's medium (MEM)

with the fluorescence reagent. Brilliant yellow-green fluorescence was seen in cytoplasm of IBRS-2 cells (Fig. 1). Control tests with the same conjugate demonstrated yellow-green fluorescence of the cytoplasm of CMK cells infected with C B5 (Fig. 2). The cultured cells infected with FMDV, LSc-2ab virus, VV or RAC-H virus showed no fluorescence as similar as noninfected controls.

Magnesium and calcium chlorides stabilized the wild SVDV strains. These strains were found to be resistant to chloroform, ethyl ether and trypsin.

Evaluation of the pathogenicity of wild SVDV strains in newborn mice

Determination of DI showed that all the SVDV strains tested have a similar pathogenicity (Table 1). Brilliant yellow-green fluorescence was observed in the impression smears of brains of the dead mice especially in the cytoplasm and processes of Purkinje cells indicating the presence of SVDV in neurons. Glial cells showed no fluorescence.

Evaluation of the homogeneity of the population of wild SVDV strains on the basis of plaque morphology

The investigated SVDV strains produced plaques 1—9 mm in diameter with proportional prevalence of plaques above 5 mm. The plaques produced

Table 2. Classification of plaques formed by wild SVDV strains

Plaque size	Diameter (mm)	Percentage formed by strains		
		SVDV/1	SVDV/2	SVDV/3
large	5—9	49	61	44
medium	3—4	17	—	40
small	1—2	34	39	16

Table 3. Pathogenicity of the clones of wild SVDV strains for newborn mice

Strain	Clone	Virus titre (log_{10})		Dose index	TPI
		$TCID_{50}$/ml	LD_{50}/ml		
SVDV/1	1—2/6*	7.0	5.8	1.2	504
	1—2/1	6.4	2.5	3.9	
	2—2/9	7.8	6.8	1.0	
SVDV/2	2—2/1	7.4	2.8	4.6	3969
	3—2/6	6.6	5.3	1.3	
SVDV/3	3—2/2	6.0	2.3	3.7	250
Control I	—	—	0.0	—	—
Control II	—	—	0.0	—	—

* The first figure denotes the SVDV strain, the scond the passage number at cloning, the third the plaque diameter (mm)
For further explanations see Table 1.

by the wild SVDV strains were classified into conventional size groups (Table 2). As results from the calculation of arithmetic means, the largest plaques were formed by the strain SVDV/2 (arithmetic mean 5.30 mm), next by the strains SVDV/1 (4.17 mm) and last by SVDV/3 (4.03 mm). The variance analysis and Duncan's test demonstrated significant differences between the arithmetic means for the strains SVDV/1-SVDV/2 and SVDV/2 - - SVDV/3. The differences between the arithmetic means for the strains SVDV/1 - SVDV/3 were not significant. Virus clones were cultured from the plaques formed by the wild SVDV strains.

Evaluation of the pathogenicity of clones of wild SVDV strains to newborn mice

Clones derived from large plaques were more virulent for baby mice than those originating from small diameter plaques (Table 3). This was confirmed by the DI and TPI. There was a high correlation between the plaque diameter and pathogenicity of the clones derived from them. For the clones of wild SVDV strains the correlation coeficient (r_{xy}) was + 0.94. IF examination of brain smears, from died baby mice showed fluorescence of the Purkinje but not of glial cells indicating that in the latter the viral antigen was absent (Figs. 3 and 4).

Discussion

The wild strain populations of various viruses are usually non-homogeneous with regard to the size of plaques formed. This general tendency was confirmed also in the case of wild SVDV strains. Comparison of the arithmetic means of the plaques formed by wild SVDV strains indicates that these strains may differ in their biological and epizootic properties.

Regarding FMDV causing symptoms similar to those produced by SVDV, there is a correlation between the arithmetic mean of the plaque size and the serological type (Parry *et al.*, 1978). Types A, C, O and SAT 1 formed the greatest number of plaques 5 mm in diameter, whereas Asia 1 and SAT 2 had a tendency to form smaller plaques (around 3 mm in diameter) and the strain SAT 3 tended to form large ones with a diameter of about 7 mm. As compared with the results of investigations on FMDV, the wild strains formed the largest number of plaques with a large diameter. It would seem, therefore, that the variants occuring in the SVDV population capable forming large diameter plaques significantly increase the degree of virulence of the whole virus population.

Interferon induction may play a certain role in various plaque size formation as proved by the studies performed on various viruses. It appeared that clones of polyoma virus obtained from plaques of small diameter, of Semliki Forest and vesicular stomatitis viruses are better interferon stimulators than clones of the same viruses derived from large plaques (Friedman *et al.*, 1963; Wagner *et al.*, 1963; Friedman and Rabson, 1964; Finter, 1967). There has been little information so far concerning the interferonogenic properties of SVDV. It seems that SVDV is a poor interferon inducer under in vitro conditions (Bakalarska-Schollenberger, personal communication). Therefore, one may suppose that other factors beside of the external ones, may significantly influence the plaque formation.

Dulbecco and Vogt (1955) elaborated the plaque size index for evaluation of strains used as vaccines. This index, however, plays no significant role, in view of the wide range of results obtained (Kańtoch and Dobrowolska, 1971; Kańtoch, 1978). According to Dubes (1956) the plaque size is determined not by one, but by several genes; it cannot, therefore, be considered for a simple genetic marker. Nevertheless, numerous authors described a correlation between plaque size and virulence of the clones isolated from them (Sabin, 1957; McClain *et al.*, 1958; Barron and Karzon, 1965; Takemoto, 1966; Clark and Wiktor, 1972; Dilovski, 1978; Cursiefen *et al.*, 1979; Malicki *et al.*, 1979). As follows from our results (Tables 1 and 3), calculation of DI and TPI seems to be especially useful for evaluation of the pathogenicity of the population of SVDV strains and their clones.

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Explanation of Micrographs (Plates XXVI—XXVII):

Fig. 1. Fluorescence of SVDV antigens in IBRS-2 cells 8 hr p.i., strain SVDV/2, ×1200.

Fig. 2. Fluorescence of CB 5 virus antigens in CMK cells 12 hr p.i., ×1200.

Fig. 3. Impression smear of the brain of suckling mouse infected with clone 2-2/9 SVDV/2; phase contrast ×340; G = glial cells; N = Purkinje cells with axonal processes.

Fig. 4. The same impression smear as in Fig. 3, direct IF, ×340.