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PLAQUE MORPHOLOGY AND PATHOGENICITY FOR NEWBORN MICE OF SWINE VESICULAR DISEASE VIRUS. II. TEMPERATURE-DEPENDENT MUTANTS AND THEIR CLONES

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Summary. — From wild swine vesicular disease virus (SVDV) strains temperature-dependent (td) mutants td 27 °C, td 32 °C and td 42 °C were derived. Differences were noted in their pathogenicity for newborn mice. The non-homogeneity of the td populations was manifested by formation of plaques of various sizes and confirmed by differential pathogenicity of the clones derived from them. A higher pathogenicity of the td mutants and their clones was associated not only with larger plaques, but also with higher temperature permissive for the given mutant. The td 27 °C mutants were not virulent for newborn mice, similarly to the attenuated SVDV strain and its clones.

Key words: *swine vesicular disease virus*; *temperature-dependent mutants*; *attenuated virus*; *clones*; *newborn mice*; *pathogenicity evaluation*

Introduction

The long known influence of temperature as one of many factors affecting variability of viruses makes sometimes possible their selective replication *in vitro* (Dubes and Wenner, 1957; Lwoff, 1959). The virulence is influenced to a high extent by in vitro passages at sub- or supraoptimal temperatures (Piraino and Hanson, 1959; Johnson, 1963; Maassab, 1965; Kańtoch, 1978). Replication temperature may be one of the factors inducing antigenic variation of viruses (Wittman *et al.*, 1965; Jablonski, 1968).

The present investigation was aimed at characterization of td 27 °C, td 32 °C, td 37 °C and td 42 °C mutants of SVDV strains and their clones based on the morphology of plaques and pathogenicity for newborn mice in comparison to the same parameters of attenuated strains and their clones.

Materials and Methods

Temperature-dependent (td) mutants. Each of three outset suspensions of SVD virus (SVDV/1, SVDV/2 and SVDV/3) was divided into four parts: pig kidney cell cultures (IBRS-2) were infected in a dose of 1—3 $TCID_{50}$/cell. The cultures were incubated at 27 °C, 32 °C, 37 °C and

Table 1. Pathogenicity for newborn mice of td mutants of SVDV strains

Strain	td mutants	Virus titre (log_{10})		DI
		$TCID_{50}$/ml	LD_{50}/ml	
SVDV/1	td 27 °C	3.2	0.0	> 3.2
	td 32 °C	7.8	2.5	5.3
	td 37 °C	8.2	3.2	5.0
	td 42 °C	7.0	3.8	3.2
SVDV/2	td 27 °C	4.5	0.0	> 4.5
	td 32 °C	7.7	3.0	4.7
	td 37 °C	8.0	3.5	4.5
	td 42 °C	6.3	4.5	1.8
SVDV/3	td 27 °C	3.0	0.0	> 3.0
	td 32 °C	7.2	1.5	5.7
	td 37 °C	8.8	3.0	5.8
	td 42 °C	6.4	3.8	2.6
Control I*	—	—	0.0	—
Control II**	—	—	0.0	—

* Uninfected ** Receiving Eagle's medium (MEM) DI = dose index

42 °C. The virus material obtained was further grown at same temperatures in IBRS-2 cultures for 20 passages.

Attenuated SVDV (SVDV/A). This strain was received from Prof. Dr. K. Malicki. The degree of its attenuation was checked in a biological test on pigs and 1-day-old baby mice (Malicki and Ladyńska, 1974).

The methods of cell culturing, plaque production, identification of viruses and their clones, biological test in newborn mice and the methods of statistical analysis have been described in our previous paper (Niemialtowski, 1983).

Results

Identification of SVDV strains and their clones

Mutants td 27 °C, td 32 °C, td 37 °C and td 42 °C derived from strains SVDV/1, SVDV/2 and SVDV/3 and their clones as well as the SVDV/A strain and its clones reacted positively with the conjugate to SVDV used for identification of wild SVDV strains and clones (Niemiałtowski, 1983). In serum neutralization test, the standard immune serum neutralized the examined strains, td mutants and clones and inhibited plaque formation.

Evaluation of the pathogenicity for newborn mice of td mutants and SVDV/A virus

It was found that the td mutants differed in their pathogenicity for newborn mice (with an exception of the td 27 °C mutants). The td 4 °C mutants exhibited the highest pathogenicity, especially those of the SVDV/2

Table 2. Pathogenicity for newborn mice of the SVDV/A strain and its clones

Strain	Clone	Virus titre (log_{10})		DI	TPI
		$TCID_{50}$/ml	LD_{50}/ml		
SVDV/A	—	6.4	0.0	> 6.4	—
	A-21/2*	6.3	0.0	> 6.3	0.6
	A-21/1	6.1	0.0	> 6.1	
Control I**	—	—	0.0	—	—
Control II***	—	—	0.0	—	—

* The letter denotes the SVDV strain, the first figure shows the number of passages in vitro, the second figure the plaque diameter (mm) ** Uninfected *** Receiving Eagle's medium (MEM) TPI = theoretical pathogenicity index (see pp. 217—222); DI = dose index

strain. All td 42 °C mutants of the tested SVDV strains showed the lowest values of the dose index (DI) as compared with the rest of td mutants. The low DI values of the td 27 °C mutants as compared with those of td 32 °C and td 37 °C mutants may have been due to the low titre of the td 27 °C mutants and the lack of virulence of these mutants for newborn mice (Table 1).

It was confirmed that the SVDV/A strain was not virulent to newborn mice (Table 2). All brain-smear samples from newborn mice infected with td mutants of SVDV and the SVDV/A strain showed fluorescence of the Purkinje cells; this was also the case with wild SVDV strains.

Table 3. Pathogenicity for newborn mice of the clones of td mutants derived from strains SVDV/1-3

Clones	Limiting value range			
	Virus titre (log_{10})		DI	TPI
	$TCID_{50}$/ml	LD_{50}/ml		
20/27 °C/2*	3.2—4.5	0.0	> 3.2— > 4.5	0.4—0.8
20/27 °C/1	2.9—4.1	0.0	> 2.9— > 4.1	
20/32 °C/3	7.0—7.8	1.5—2.8	4.8— 5.5	23.7—629
20/32 °C/1	6.9—7.6	0.0—0.5	> 6.9— > 7.6	
20/37 °C/3	7.8—8.9	3.2—3.8	4.0— 5.7	247—430
20/37 °C/1	7.9—8.6	0.5	7.4— 8.1	
20/42 °C/5	6.3—7.2	3.8—4.8	1.5— 3.4	394—11 100
20/42 °C/1	6.0—7.0	0.5—1.0	≧5.5— 6.0	
Control I**	—	0.0	—	—
Control II***	—	0.0	—	—

* The first number denotes passages of the td mutant, the second the temperature, the third the plaque diameter (in mm).
For furhter explanations see Table 2.

Evaluation of homogeneity of the td mutant populations and of SVDV/A based on plaque morphology

Studied populations proved nonhomogeneous regarding to the plaque size. With the rise of temperature, the percentage of large diameter plaques increased. The largest plaques were formed by the td mutants 42 °C (arithmetic means 2.64 mm, 2.40 mm and 1.92 mm); medium by td 37 °C mutants (2.33 mm, 1.83 mm and 1.78 mm); then followed the td 32 °C mutants (1.48 mm, 1.36 m and 1.46 mm) and at last the td 27 °C mutants (1.19 mm, 1.12 mm and 1.15 mm). Variance analysis and Duncan's test demonstrated significant differences between all the arithmetic means of plaque size formed by td mutants of SVDV strains except of the difference between td 37 °C and td 42 °C mutants for the SVDV/3 strain. The SVDV/A strain formed plaques either 1 mm (89%) or 2 mm in diameter (11%), the arithmetic mean being 1.1 mm.

Evaluation of the pathogenicity of the clones of td mutants and of SVDV/A for newborn mice

Clones of td mutants of SVDV were virulent for newborn mice (Table 3). The DI values were lowest for the most virulent SVDV populations, i.e. for td 42 °C mutants, followed by td 37 °C and td 32 °C mutants (with some exceptions). The relatively low DI values for td 27 °C mutants cannot be compared with those for the most of td mutants due to the low titres of the td 27 °C mutants. A high correlation was found between the plaque diameter and the pathogenecity of the clones obtained from it. The correlation coefficient (r_{xy}) was + 0.80 for clones of td mutants. Clones of the SVDV/A strain were not virulent for newborn mice (Table 2).

The brains of newborn mice infected with different clones of td mutants and SVDV/A showed positive fluorescence of Purkinje cells in direct immunofluorescence proving the presence of SVDV.

Discussion

Virulence of viruses is greatly influenced by passages *in vitro* at sub- or supraoptimal temperatures (Dubes and Wenner, 1957; Lwoff, 1959; Piraino and Hanson, 1959; Johnson, 1963; Maassab, 1965). Among SVDV mutants td 42 °C, td 37 °C and 32 °C a partial loss of virulence was observed as compared with that of wild strains; td 27 °C mutants showed no virulence at all. This was due to the influence of various temperatures on the virulence of viruses pasaged *in vitro*. In plaques of the same diameter formed by uncloned populations of more virulent td mutants, virions raising large plaques quantitatively prevailed. It is not certain whether SVDV replication at 27 °C caused complete attenuation (the titre of td 27 °C mutants greatly differed from those of the remaining td mutants when newborn mice were infected) and whether this observation is of any practical significance.

Preston and Garland (1979) established that the virulence of SVDV was not dependent on sensitivity of the virus to a definite temperature. SVDV

strains represent probably a mixture of particles varying in virulence, as similar as poliovirus strains (Sabin, 1955; 1957). It seems that there is no direct relationship between the size of the plaques from which various SVDV clones were isolated and their pathogenicity for newborn mice. This is confirmed by the fact that clones of wild SVDV strains (Niemiałtowski, 1983) and various td mutants isolated from plaques of same diameter were either virulent or avirulent for newborn mice depending on the number and temperature of passages.

Noteworthy is the presence of SVDV antigens in Purkinje cells of newborn mice brains, 20 days p.i. with the td 27 °C mutants and with SVDV/A. In the case of the td 27 °C mutants this may prove their total attenuation by replication at 27 °C.

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References

Dubes, G. R., and Wenner, H. A. (1957): Virulence of polioviruses in relation to variant characteristics distinguishable on cells in vitro. *Virology* **4**, 275—296.

Jablonski, L. (1968): Current questions concerning genetics of animal viruses (in Polish). *Post. Mikrob.* **7**, 85—102.

Johnson, H. N. (1963): Selection of a variant of western encephalitis virus of low pathogenicity for study as a line virus vaccine. *Amer. J. trop. Med. Hyg.* **12**, 604—610.

Kańtoch, M. (1978): Markers and vaccines. *Advanc. Virus Res.* **22**, 259—325.

Lwoff, A. (1959): Factors influencing the evolution of viral diseases at the cellular level and in the organism. *Bact. Rev.* **23**, 109—124.

Malicki, K., and Ladyńska, A. (1974): Range of specific sensitivity in vitro of cells to infection with the swine vesicular disease virus (in Polish), p. 429. *In Bulletin of V Conference of Polish Society of Veterinary Sciences*, Olsztyn, 12—14 September 1974, Poland.

Maassab, H. F. (1965): Adaptation and growth characteristics of influenza virus at 25 °C. *Nature (Lond.)* **213**, 612—614.

Niemialtowski, M. (1983): Plaque morphology and pathogenicity for newborn mice of swine vesicular virus. I. Wild strains and their clones. *Acta virol.* **27**, 217—222.

Piraino, F., and Hanson, R. P. (1959): Isolaion of a nonneurotropic line of Newcastle disease virus from a neurotropic parental type. *Virology* **8**, 383—385.

Preston, K. J., and Garland, A. J. M. (1979): In vivo and in vitro studies on temperature-sensitive mutants of swine vesicular disease virus. *J. Hyg. (Camb.)* **83**, 319—330.

Sabin, A. B. (1955): Behavior of chimpanzee-avirulent poliomyelitis viruses in experimentally infected human volunteers. *Amer. J. Med. Sci.* **230**, 1—8.

Sabin, A. B. (1957): Properties of attenuated polioviruses and their behavior in human beings. *Ann. N. Y. Acad. Sci. Spec. Publ.* **5**, 113—140.

Wittman, H. G., Wittman-Liebold, B., and Jaurequil-Adell, J. (1965): Primary protein structure of the temperature-sensitive mutant of the tobacco virus. I. Spontaneous mutants (in German). *Z. Naturforsch.* **20**b, 1224—1234.