0022-538X/78/0025-0446$02.00/0
JOURNAL OF VIROLOGY, Jan. 1978, p. 446-449

Vol. 25, No. 1
Printed in U.S.A.

Classification of the New Jersey Serotype of Vesicular Stomatitis Virus into Two Subtypes

M. E. REICHMANN,[1] W. M. SCHNITZLEIN,[1] DAVID H. L. BISHOP,[2] R. A. LAZZERINI,[3] SARA T. BEATRICE,[4] AND ROBERT R. WAGNER[4]*

Departments of Microbiology at the University of Illinois, Urbana, Illinois 61803,[1] University of Alabama, Birmingham, Alabama 35294,[2] and University of Virginia, Charlottesville, Virginia 22901[4]; and the Laboratory of Molecular Biology, National Institute of Neurological and Communicative Diseases and Stroke, Bethesda, Maryland 20014[3]

Received for publication 7 September 1977

We propose a reclassification of five strains of the New Jersey serotype of vesicular stomatitis virus into two subtypes designated Concan and Hazelhurst. This subclassification into two subtypes is based on reciprocal differences in antibody neutralization of virion infectivity, nucleotide base sequence homology, oligonucleotide maps of virion RNA, and interference by defective-interfering particles.

Vesicular stomatitis (VS) viruses, the prototype members of the rhabdovirus group (20), were originally isolated during widespread outbreaks of disease in cattle, horses, and swine (8). These isolates were classified into two serotypes, Indiana (VS_{Ind}) and New Jersey (VS_{NJ}), based on little or no reciprocal cross-neutralization of infectivity (2). The Indiana serotype has been further subdivided into four distinct but antigenically related subtypes, Indiana, Argentina, Brazil, and Cocal; (2, 7), but all strains of the New Jersey serotype have been considered, until now, to belong to a single antigenically homogeneous type. It is now known that the major antigenic determinant is the virion glycoprotein, which gives rise to and reacts with neutralizing antibody (10). Heterotypic interference by defective-interfering (DI) particles are consistent with and partially confirm the serological classifications of VS virus (6). However, cross-hybridization studies with virion RNA and mRNA from infected cells revealed much greater disparity in genetic relatedness of different VS virus strains; for example, the antigenically related Indiana and Cocal subtypes of the VS_{Ind} serotype exhibit only 10% base sequence homology by reciprocal annealing (15).

During recent mapping studies of DI-particle RNA, it was found that all isolates of the Indiana subtype of the VS_{Ind} serotype exhibited 90 to 100% base sequence homology (12, 16, 19). However, VS_{NJ} strains Ogden and Missouri showed RNA base sequence homology of only 25% compared to >90% homology between RNAs of the Ogden and Concan strains (17). Most investigators appear to use Ogden and Concan VS_{NJ} wild-type virus, although all current temperature-sensitive (*ts*) mutants of VS_{NJ} virus are derived from the Glasgow passage of the Missouri strain (14). To minimize confusion in comparing data emanating from different laboratories, it seemed essential to determine the degree of relatedness among standard isolates of the New Jersey serotype of VS virus. Reported here are comparative data on five VS_{NJ} virus strains which indicate that they can be grouped into two quite distinct subtypes based on four independent parameters: cross-neutralization of infectivity, cross-hybridization of RNAs, virion RNA oligonucleotide mapping, and DI-particle interference.

Table 1 summarizes the origin and history of the five strains of VS_{NJ} virus used in this study; the strains are designated Concan, Ogden, Guatemala, Missouri, and Hazelhurst to identify the city, state, or country where they were isolated. Also indicated are the investigator and/or the laboratory selected to contribute each representative isolate. Unfortunately, the Concan isolate had two contributors, hence, the designations Concan (Prevec) and Concan (Wagner).

Table 2 compares the reciprocal cross-neutralization of the infectivity of the five designated strains of VS_{NJ} virus by their respective antisera as well as that of the heterologous serotype, VS_{Ind} virus (San Juan strain). Quite clearly, none of the five VS_{NJ} strains shares significant type-specific antigens with the VS_{Ind} serotype. Reciprocal neutralization among the five VS_{NJ} strains revealed considerable cross-reactivity, to a degree that readily permits relegating all five VS_{NJ} isolates to the same serotype. However, these cross-neutralizations indicate a basis for segregation of these five strains into two

TABLE 1. *Designations and origins of five strains within the New Jersey serotype of VS virus*

Strain		Isolation History Summary				
Designation	Donor	Year	Animal	Place	Investigator	Reference
Concan[a]	Wagner Prevec	1949	Cattle	Texas	Mott	9
Ogden[b]	Holland	1949	Cattle Horse	Utah	Mott	8
Guatemala[c]	Bishop	1970	Mosquito	Guatemala	Calisher	
Missouri[d]	Pringle	1943	Swine	Kansas City, Mo.	Brooksby	1, 9, 18
Hazelhurst[e]	ATCC	1952	Swine	Georgia	Holbrook	19

[a] Isolated in Concan, Tex., from the dental pad of an infected cow and subsequently passed three times in cows and four times in embryonated eggs before receipt by Wagner from U.S. Department of Agriculture, Beltsville, Md.

[b] Obtained by J. J. Holland from F. Schaffer in Berkeley, Calif., who traced it back via S. Madin to L. O. Mott of the U.S. Department of Agriculture, Beltsville, Md., who, according to R. P. Hanson (9), isolated the virus from the tongue epithelial tissue of a cow on the Isaacson farm in Ogden, Utah.

[c] Isolated from a pool of 95 unfed mosquitoes (*Culex nigripalpus*) at Montufar, Guatemala, and cloned in BHK-21 cells in Birmingham, Ala., after three suckling mouse brain passages at the Center for Disease Control, Fort Collins, Colo. (C. H. Calisher, personal communication).

[d] Isolated by J. F. Brooksby (1) from infected swine ostensibly in Kansas City, Mo. This isolate has been passaged extensively in Glasgow and without cloning in BHK-21 cells in Urbana, Ill.

[e] Isolated from epithelial tissue of swine in Hazelhurst, Ga., and deposited in the American Type Culture Collection (ATCC 159).

TABLE 2. *Cross-neutralization of five cloned isolates of the New Jersey serotype of VS virus with antisera against each isolate*[a]

Virus isolate	Antibody dilution ($\times 10^{-3}$) resulting in 50% plaque reduction					
	Concan	Guatemala	Ogden	Missouri	Hazelhurst	Indiana
Concan[b]	50	5	10	25	5	<0.01
Guatemala	50	10	10	25	5	<0.01
Ogden	10	5	5	25	5	<0.01
Missouri	0.1	2.5	2.5	50	5	<0.01
Hazelhurst	0.1	2.5	2.5	25	25	<0.01
Indiana	<0.01	<0.01	<0.01	<0.01	<0.01	25

[a] Each VS_{NJ} strain was plaque purified, and clones were grown in BHK-21 cells to titers of 2×10^8 to 8×10^8 PFU/ml. Antibody was raised by three footpad or intracutaneous injections of rabbits with 0.5 mg of each UV-irradiated, purified stock preparation in complete Freund adjuvant at intervals of 4 weeks. Sera from bleedings at 7 days after the last injection were inactivated at 56°C for 1 h. Dilutions of each antiserum were tested for capacity to neutralize 100 and 1,000 PFU of each VS_{NJ} virus strain, as well as the VS_{Ind} virus (San Juan strain). End points of plaque neutralization are recorded as the dilution at which each antiserum reduced plaque titers by 50%.

[b] Concan isolate from Wagner's laboratory.

subtypes. This is most clearly demonstrated with anti-Concan serum, which neutralized the Concan, Guatemala, and Ogden strains 100 to 500 times more efficiently than it neutralized the Missouri and Hazelhurst strains. However, the four other antisera provided somewhat less compelling evidence for dividing VS_{NJ} virus isolates into two distinct subtypes; VS_{NJ} antisera other than anti-Concan had homologous neutralizing titers only two- to fivefold greater than their heterologous neutralizing activity. In the case of Ogden and Missouri antisera, differential neutralization of heterologous strains was not significant. Despite this evidence for unidirectional antigenic divergence, it seems quite reasonable to divide the five VS_{NJ} strains into two subtypes represented by Concan, Guatemala, and Ogden as distinct from Missouri and Hazelhurst.

Cross-hybridization studies were performed with purified mRNA species and viral RNAs prepared from BHK-21(C13) cells infected by each of these isolates, as described previously (16). Table 3 shows results of reciprocal annealing experiments in which radioactively labeled 13–18S and 30S mRNA species of Ogden, Missouri, or Concan were tested with various nonradioactive virion 42S RNAs. The data express the extent of anealing in terms of percent radioactive counts resistant to pancreatic and T_1 RNase digestion. The isolates again seem to fall into two subtypes by virtue of their anneal-

TABLE 3. *Cross-hybridization of virion RNA and mRNA among different strains of New Jersey serotype*

Virion RNA	% mRNA resistant to RNase[a]					
	Ogden		Concan (Prevec)		Missouri	
	13-18S	30S	13-18S	30S	13-18S	30S
Ogden	99	94	91	80		
Concan (Wagner)	93	92			33	33
Concan (Prevec)			97	86		
Guatemala	85	87			29	31
Missouri	25	19	24	16	94	88
Hazelhurst	31	24			82	77
No RNA	1	5	0	3	1	14
BHK rRNA	2	5			1	14

[a] Unlabeled virion and rRNAs were annealed to 0.9×10^3 to 1.2×10^3 cpm of ^{3}H-labeled viral 13-18S and 30S RNA from infected cells. Annealed mixtures were digested with RNases A and T_1, as previously described (16, 17).

ing properties: subtype 1—Ogden, Concan, and Guatemala; and subtype 2—Hazelhurst and Missouri. Members within the same subtype exhibit RNA homologies of 80 to 100%, whereas members of different subtypes cross-anneal to an extent not exceeding 30%.

Oligonucleotide fingerprints of T_1 RNase digests are, in general, consistent with these conclusions (3). Figure 1 shows a tracing of the region corresponding to the large, and presumably unique, oligonucleotides separated by two-dimensional electrophoresis in polyacrylamide slab gels (3). The patterns obtained with Ogden (Fig. 1a) and Concan (not shown) RNAs are virtually identical, but Guatemala RNA patterns, while similar, differ in a number of oligonucleotides (3). On the other hand, Missouri oligonucleotide patterns (Fig. 1b) are very different. The crosses in Fig. 1a and b indicate the position of ink markers, which were used to obtain reproducible patterns. This method for comparing isolates is, however, much more subjective and time consuming than reciprocal annealing. Moreover, it is probably too sensitive, since it often reflects differences that do not necessarily originate from large regions of nonhomology (3).

Some preliminary data indicate that the two subtypes also differ in biological properties. It has been shown previously that the DI particles generated by a heat-resistant (HR) mutant of the Indiana serotype interfered heterotypically with the New Jersey serotype of VS virus (13). These experiments were originally performed with the Concan isolate. When heterotypic interfering ability of the HR DI particle was measured with Hazelhurst, the results shown in Fig. 2 were obtained. As previously reported (11), infections with Concan and Indiana were interfered with equally efficiently. However, no interference with Hazelhurst took place.

Cross-hybridization, oligonucleotide mapping, and autointerference consistent with differential cross-neutralization justify a subdivision of presently available VS_{NJ} virus isolates into two subtypes. To provide a name for each subgroup, a laboratory strain of reference has to be defined. Historically, the oldest New Jersey isolate is the so-called laboratory strain isolated from cattle

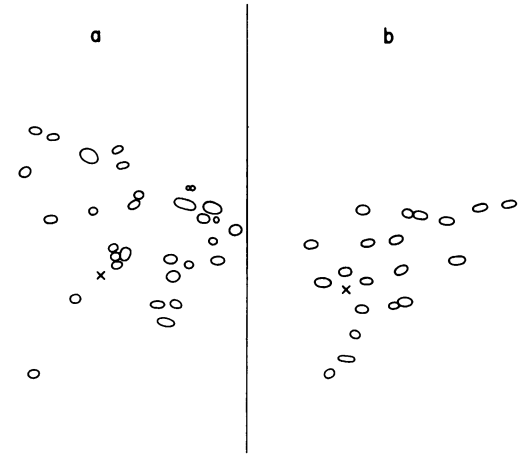

FIG. 1. *Tracings of large oligonucleotides obtained by two-dimensional electrophoresis of T1 RNase-digested viral RNAs. Viral RNAs were digested and subjected to electrophoresis as described previously (3). (a) Ogden; (b) Missouri.*

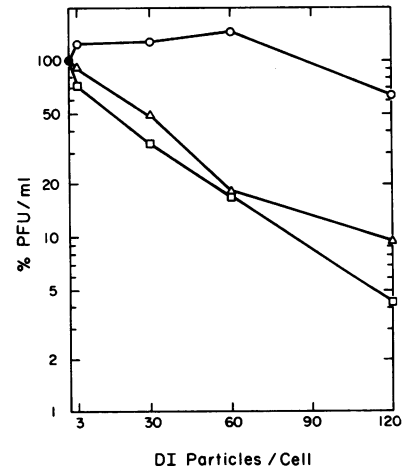

FIG. 2. *Homotypic and heterotypic interference by HR (Indiana) DI particles. Experimental conditions were as described previously (11). Results are plotted in percent yields of infectious virus as a function of HR DI-particle concentration of the inoculum. Symbols:* (○) *Hazelhurst;* (△) *Concan;* (□) *Indiana.*

by W. E. Cotton (4, 5). Unfortunately, this isolate could not be obtained. The Ogden isolate is the oldest one in our collection, but its history suffers a serious gap between 1949 and 1952, which has already been discussed. Moreover, in the earliest reference in the literature the isolate was designated as 48 Ogden (8), where 48 signifies the year of isolation (Schaffer, personal communication). However, the Ogden outbreak clearly dates back to 1949 (9). On the other hand, the records of the Concan isolate are continuous, going back to the cow from which it was isolated during the Texas outbreak. We therefore choose this isolate as the laboratory reference strain and suggest naming the subtype Concan. The availability of Hazelhurst through the American Type Culture Collection to any research laboratory would make it convenient to define it as the laboratory reference strain and name the second subtype Hazelhurst.

It should be noted that all isolates of the Concan subtype originated from outbreaks in horses and cattle, whereas those of the Hazelhurst subtype occurred in swine. There were several similar outbreaks between 1952 and 1955, which affected swine only. Several isolates were available at the U.S. Department of Agriculture, Parasite Research Branch, Beltsville, Md., in 1956 (11). These specimens were later moved to the U.S. Department of Agriculture, National Animal Disease Laboratory, Ames, Iowa, where they were finally discarded because of shortage of storage space. In spite of a concerted effort by several of us, we were unable to obtain any of these or later U.S. isolates originating from outbreaks in swine only. It was, therefore, impossible to ascertain whether the host difference exhibited by the two subtypes is purely fortuitous.

It is very likely that future tests of VS virus New Jersey serotype isolates in some laboratory will turn up specimens that will not fit either of the two proposed subtypes. It might then become necessary to either define new additional subtypes or completely reclassify these isolates.

This study was supported by Public Health Service grants AI 12070, AI 13402, and AI 11112 from the National Institute of Allergy and Infectious Diseases, by National Science Foundation grant BMS-72-2223, and by American Cancer Society grant VC-88.

LITERATURE CITED

1. **Brooksby, J. B.** 1948. Vesicular stomatitis and foot-and-mouth disease differentiation by complement-fixation. Proc. Soc. Exp. Med. **67**:254–258.
2. **Cartwright, B., and F. Brown.** 1972. Serological relationships between different strains of vesicular stomatitis virus. J. Gen. Virol. **16**:391–398.
3. **Clewley, J. P., D. H. L. Bishop, C. Y. Kang, J. Coffin, W. M. Schnitzlein, M. E. Reichmann, and R. E. Shope.** 1977. Oligonucleotide fingerprints of RNA species obtained from rhabdoviruses belonging to the vesicular stomatitis virus subgroup. J. Virol. **23**:152–166.
4. **Cotton, W. E.** 1927. Vesicular stomatitis. Vet. Med. **22**:169–175.
5. **Cotton, W. E.** 1927. The causal agent of vesicular stomatitis proved to be a filter-passing virus. Am. Vet. Med. Assoc. J. **23**:168–181.
6. **Crick, J., and F. Brown.** 1972. Interference as a measure of cross relationship in the vesicular stomatitis group of rhabdoviruses. J. Gen. Virol. **18**:79–82.
7. **Federer, K. B., R. Burrows, and J. B. Brooksby.** 1967. Vesicular stomatitis virus—the relationship between some strains of the Indiana serotype. Res. Vet. Sci. **8**:103–117.
8. **Hanson, R. P.** 1952. The natural history of vesicular stomatitis. Bacteriol. Rev. **16**:179–204.
9. **Holbrook, A. A., J. N. Geleta, and W. C. Patterson.** 1956. A typing study of vesicular stomatitis virus field samples of swine origin, p. 293–301. *In* Proceedings of the U.S. Livestock Sanitation Association, 60th Annual Meeting.
10. **Kelley, J. M., S. U. Emerson, and R. R. Wagner.** 1972. The glycoprotein of vesicular stomatitis virus is the antigen that gives rise to and reacts with neutralizing antibodies. J. Virol. **10**:1231–1235.
11. **Khan, S. R., and R. A. Lazzarini.** 1977. The relationship between autointerference and the replication of a defective interfering particle. Virology **77**:189–201.
12. **Leamnson, R. N., and M. E. Reichmann.** 1974. The RNA of defective vesicular stomatitis virus particles in relation to viral cistrons. J. Mol. Biol. **85**:551–568.
13. **Prevec, L., and C. Y. Kang.** 1970. Homotypic and heterotypic interference by defective particles of vesicular stomatitis virus. Nature (London) **228**:25–27.
14. **Pringle, C. R., I. B. Duncan, and M. Stevenson.** 1971. Isolation and characterization of temperature sensitive mutants of vesicular stomatitis virus, New Jersey serotype. J. Virol. **8**:836–841.
15. **Repik, P., A. Flamand, H. F. Clark, J. F. Obijeski, P. Roy, and D. H. L. Bishop.** 1974. Detection of homologous RNA sequences among six rhabdovirus genomes. J. Virol. **13**:250–252.
16. **Schnitzlein, W. M., and M. E. Reichmann.** 1976. The size and cistronic origin of defective vesicular stomatitis virus particles RNAs in relation to homotypic and heterotypic interference. J. Mol. Biol. **101**:307–325.
17. **Schnitzlein, W. M., and M. E. Reichmann.** 1977. Interference and RNA homologies of New Jersey serotype isolates of vesicular stomatitis virus and their defective particles. Virology **80**:275–288.
18. **Shahan, M. S., A. H. Frank, and L. O. Mott.** 1946. Studies of vesicular stomatitis with special reference to a virus of swine origin. J. Am. Vet. Med. Assoc. **108**:5–19.
19. **Stamminger, G., and R. A. Lazzarini.** 1974. Analysis of the RNA of defective VSV particles. Cell **3**:85–93.
20. **Wagner, R. R.** 1975. Reproduction of rhabdoviruses, p. 1–92. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 4. Plenum Press, New York.