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

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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 (VSIw) and New Jersey (VSNJ), 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 Cocalal; (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 VSIw 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 VSIw serotype exhibited 90 to 100% base sequence homology (12, 16, 19). However, VSNJ 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 VSNJ wild-type virus, although all current temperature-sensitive (ts) mutants of VSNJ 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 VSNJ 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 VSNJ 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 VSNJ virus by their respective antisera as well as that of the heterologous serotype, VSIw virus (San Juan strain). Quite clearly, none of the five VSNJ strains shares significant type-specific antigens with the VSIw serotype. Reciprocal neutralization among the five VSNJ strains revealed considerable cross-reactivity, to a degree that readily permits relegating all five VSNJ isolates to the same serotype. However, these cross-neutralizations indicate a basis for segregation of these five strains into two
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_{N_J} virus isolates into two distinct subtypes; VS_{N_J} 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_{N_J} 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 non-radioactive virion 42S RNAs. The data express the extent of annealing 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

<table>
<thead>
<tr>
<th>Virion RNA</th>
<th>Ogden (Wagner)</th>
<th>Ogden (Prevec)</th>
<th>Concan (Prevec)</th>
<th>Missouri</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13-18S 30S</td>
<td>13-18S 30S</td>
<td>13-18S 30S</td>
<td></td>
</tr>
<tr>
<td>Ogden</td>
<td>29</td>
<td>24</td>
<td>91</td>
<td>80</td>
</tr>
<tr>
<td>Concan (Wagner)</td>
<td>93</td>
<td>87</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Concan (Prevec)</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>86</td>
</tr>
<tr>
<td>Guatemala</td>
<td>85</td>
<td>87</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Missouri</td>
<td>25</td>
<td>19</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Hazelhurst</td>
<td>31</td>
<td>24</td>
<td>24</td>
<td>77</td>
</tr>
<tr>
<td>No RNA</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>BHK rRNA</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*Unlabeled virion and RNA's were annealed to 0.9 × 10^4 to 1.2 × 10^5 cpn of 3H-labeled viral 13-18S and 30S RNA from infected cells. Annealed mixtures were digested with RNases A and T1, 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 T1 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 non-homology (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	n 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 4g Ogden (8), where 4g 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.

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LITERATURE CITED

NOTES


