Collaborative Study of Temperature-Sensitive Mutants of Herpes Simplex Virus Type 2

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Eighteen complementation groups were identified by complementation tests and by phenotype from twenty temperature-sensitive mutants isolated independently in Glasgow, Scotland, and Houston, Tex.

This report describes the results of a collaborative study to compare, by complementation, temperature-sensitive (*ts*) mutants of type 2 herpes simplex virus (HSV-2) that had been isolated independently in Glasgow, Scotland, and Houston, Tex. Twelve Glasgow mutants that had previously been shown to belong to different complementation groups (4a, 7) were compared to eight Houston mutants that had also been shown to belong to separate complementation groups (3). Three types of complementation test were used to compare the mutants: the qualitative test (2), the quantitative test (6), and the infectious center test (7). These tests were carried out as described previously except that Vero cells and a multiplicity of infection of 2.5 were used for the quantitative test and that monolayers instead of suspended cells were used to plate the infectious center test. The formulae (1) used for calculation of complementation indices (CI) were, in the quantitative test:

$$CI = \frac{(X + Y)^{34 C} - (X + Y)^{38 C}}{X^{34 C} + Y^{34 C}} \quad (i)$$

and, in the infectious center test:

$$CI = \frac{(X + Y)^{38 C/32 C}}{X^{38 C/32 C} + Y^{38 C/32 C}} \quad (ii)$$

X and Y represent in (i) the yield and in (ii) the number of infectious centers from cells infected mixedly or singly with the two *ts* mutants, X and Y. A value of 2.0 or more in the CI was regarded as evidence of complementation.

The Glasgow wild-type virus from which the mutants had been derived was different from that used for isolation of the Houston mutants. Stocks of the Glasgow mutants had been prepared in BHK-21 cells, but titration in Vero cells showed titers similar to those obtained in

BHK-21 cells. Although their different origin and adaptation to different cells might have hindered complementation between the two sets of mutants, in fact most complemented well. The results of tests on eight Glasgow mutants which complemented all eight Houston mutants are shown in Table 1. Occasional negative results were usually disregarded, as this is known to happen with mutants that normally complement (7). Complementation was, on the whole, relatively efficient, and many of the crosses showed CIs of more than 50. Nevertheless, negative results were obtained in several quantitative tests with *ts8* and twice with *ts12* but, since there were at least two positive results in other tests with the same crosses, *ts8* and *ts12* were regarded as showing complementation with all eight Houston mutants. Virus yields obtained in four crosses that showed different CIs in the same quantitative tests are shown in Table 2.

The other four Glasgow mutants gave negative results with at least one Houston mutant (Table 3). Negative results were obtained in two crosses in which the pairs of mutants involved were phenotypically similar. The Glasgow mutant was therefore placed in the same group as its Houston counterpart, and this defined two complementation groups: one containing *ts6* and *tsB5* and the other containing *ts10* and *tsC2*. *ts9* also failed to complement *tsB5*, although it does complement *ts6* (7). However, it differs from these two mutants phenotypically (Table 4) and, although the possibility that *tsB5* is a double mutant has not been ruled out, it has been provisionally placed in a different group from *ts6* and *tsB5*. *ts2* and *tsD6* also gave generally negative results—the positive mean in the quantitative test is based on only one positive result. But since *ts2* is DNA negative and *tsD6* is DNA positive, they were put in separate groups. Most of the other crosses showed positive complementation, but five gave negative quantitative tests and comple-

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TABLE 1. *Qualitative and quantitative tests on Glasgow and Houston mutants showing positive complementation*

Houston <i>ts</i> mutants	Complementation							
	1 ^a	3	4	5	8	11	12	13
A8	⁺ ^b 440 ^c	+	+	+	+	+	++	+
		61.0	33.2	43.9	0.6; 2.0 <i>15.5</i> ^d	43.8	9.3; 5.6	54.1
B5	+	+	+	+	+	+	++	++
	558	390	53.5	387	1.8; 1.5 <i>3.6</i>	9,750	19.3; 2.5	2.0; 3.8 154
C2	+	+	+	+	+	+	++	++
	18.7	93.5	27.1	322	1.2; 13.4 <i>0.8</i>	193	8.4; 9.5	1,872
D6	+	+	+	+	+	+	+	+
	862	49.0	36.2	104.9	0.4; 7.8 <i>2.3</i>	1,358	30.3	85.7
E7	+	+	+	+	+	+	++	+
	820	33.3	66.7	506	1.8; 3.2 <i>4.4</i>	333	4.0; 2.6	43
F3	+	+	+	+	+	+	++	+
	22.1	179	114	38.4	5.7; 47.6	414	1.6; 29.9 50.8	8.1; 3.5
G4	+	+	+	+	+	+	+	++
	1,111	311	93.5	567	3.1; 9.1	515	78.6	59.3
H9	+	+	+	+	+	+	++	+
	57.3	5.2	5.2	7.4; 10.8	1.0; 3.7 <i>128</i>	41.8	2.2; 0.5; 6.0; 4.7	12.4

^a Glasgow *ts* mutants.^b +, Positive result in qualitative test; ++, positive results in two qualitative tests.^c Complementation index from quantitative complementation test; values of 2 or greater are considered to be positive.^d Italicized numbers indicate complementation index from infectious center assay; values of 2 or greater are considered to be positive.TABLE 2. *Yield of virus (PFU/ml) in four crosses in one quantitative test*

Mutants	Mixed infection		Singly infected controls						CI
	38 C	34 C	Mutant	38 C	34 C	Mutant	38 C	34 C	
<i>ts1</i> + <i>tsD6</i>	4.8×10^4	1.6×10^5	<i>ts1</i>	0	7.5×10^1	<i>tsD6</i>	0	5.5×10^1	862
<i>ts5</i> + <i>tsF3</i>	5.4×10^3	3.5×10^4	<i>ts5</i>	0	1.5×10^1	<i>tsF3</i>	0	1.4×10^3	38.4
<i>ts9</i> + <i>tsF3</i>	4.0×10^2	8.9×10^3	<i>ts9</i>	0	0	<i>tsF3</i>	0	1.4×10^3	6.1
<i>ts6</i> + <i>tsB5</i>	0	2.4×10^2	<i>ts6</i>	0	6.5×10^1	<i>tsB5</i>	0	8.0×10^1	1.7

mentation was detected by qualitative and infectious center tests (*ts2* with *tsB5* and *tsC2*; *ts6* with *tsC2* and *tsD6*; *ts9* with *tsE7*).

Altogether 18 complementation groups were defined as a result of the tests (Table 4), which confirmed the usefulness of the qualitative test and showed that it correlates well with the infectious center test. Both were more sensitive in detecting complementation than the quantitative test, possibly because they involve multi-

ple rounds of viral replication, whereas the quantitative test measures the yield from one cycle of virus growth. The low yields from cells infected with HSV-2 may be the reason for the apparent lack of sensitivity of the quantitative test. Although there were discrepancies between the qualitative and infectious center tests on the one hand and the quantitative test on the other, and although there were almost certainly recombinants in the progeny from

TABLE 3. Results of complementation tests on mutants showing negative or variable complementation

Glasgow mutants	Complementation							
	A8 ^a	B5	C2	D6	E7	F3	G4	H9
<i>ts2</i>	⁺ ^b 3.4 ^{c,d}	++ 0.7 79 ^e	++ 0.7 18.9	— ^b 3.2 1.6	+	+	++ 12.0 ^e	++ 2.3 227
<i>ts6</i>	— 5.3	— 1.7 0.3	++ 1.6 17.4	++ 1.3 66.7	+	+	+	— 7.7
<i>ts9</i>	+	— 1.1 0.4	+ 2.8 13.2	++ 12.2	+ 1.6 49.3	++ 6.1	+	+
<i>ts10</i>	+	+	— 1.5 0.1	+	+	+	+	+
	29.8 ^d	164 ^d		34.2 ^d	120 ^d	22.9 ^d	142 ^d	15.1 13.3

^a Houston mutants.^b Indicates positive (+) or negative (—) qualitative test; two symbols indicate the results of repeat qualitative tests.^c Complementation index from quantitative test; results are based on the mean of at least two results except as noted.^d Results of one test only.^e Italicized numbers indicate CI from infectious center test; results are based on the mean of at least two results.TABLE 4. Complementation group and *ts* mutants of HSV-2

Complementation group no.	<i>ts</i> mutants		Phenotype (38 C) ^a	
	Glasgow	Houston	Viral DNA	Viral DNA polymerase
1	<i>ts1</i>		—	++
2		<i>tsH9</i>	—	—
3	<i>ts6</i>	<i>tsB5</i>	—	— ^b
4	<i>ts9</i>		—	—
5	<i>ts10</i>	<i>tsC2</i>	—	±
6	<i>ts11</i>		—	±
7		<i>tsA8</i>	—	±
8	<i>ts2</i>		—	+
9	<i>ts8</i>		—	+
10	<i>ts3</i>		+	+
11	<i>ts4</i>		+	+
12	<i>ts5</i>		+	+
13	<i>ts12</i>		+	+
14	<i>ts13</i>		+	+
15		<i>tsD6</i>	+	+
16		<i>tsE7</i>	+	+
17		<i>tsF3</i>	+	+
18		<i>tsG4</i>	+	+

^a Viral DNA and DNA polymerase phenotypes are from references 3, 4, 4a, and 5 and J. Hay, H. Moss, A. T. Jamieson, and M. C. Timbury, *J. Gen. Virol.*, in press.^b Enzyme is thermolabile in vivo.

both qualitative and infectious center tests, we feel that these tests are valid indicators of complementation. Plaques formed in infectious center tests have been analyzed and found to con-

tain infectious virus that included both recombinants (in 45% of the plaques sampled) and parental *ts* genotypes (L. Wilson, personal communication). The presence of the parental *ts* genotypes proved that complementation had taken place.

A new scheme for numbering the complementation groups of *ts* mutants of HSV-2 is shown in Table 4. Numbers have been used rather than letters because of the large coding potential of the virus. If a similar system were later applied to HSV-1, a prefix could be added to the number of the complementation group to indicate the type of virus to which it refers. The large coding potential also indicates that the 18 complementation groups shown represent only a small proportion of the total number of possible genes of this virus.

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