INFECTION AND IMMUNITY, Aug. 1974, p. 400-401
Vol. 10, No. 2

Printed in U.S.A.

Linkage Between Reproductive Capacity at Increased Temperature and Neurotropic Activity in A/NWS HONi (H_oN_1) Influenza Virus

JAROSLAVA SVOBODOVÁ, EVA TUČKOVÁ, AND VLADIMÍR VONKA

Department of Experimental Virology, Institute of Sera and Vaccines, Prague, Czechoslovakia

Received for publication 7 December 1973

We investigated the rate of covariation between the ability of the NWS strain of influenza virus to reproduce at increased temperature and its neurotropic properties. Results indicated that increased growth at 39 C was related to increased neurovirulence, and acquisition of the temperature-sensitive character was related to loss of neurovirulence.

It has been repeatedly demonstrated that cold-adapted or thermosensitive (ts) mutants of influenza viruses display decreased virulence after intranasal administration to mice or hamsters (6, 7, 9) or chicken embryos (8). Although avirulent, these viruses replicate sufficiently in the infected animals to induce immunity to challenge with virulent homologous virus (6, 7, 9). At present, ts mutants of influenza viruses are under consideration as possible candidate viruses for live influenza vaccines.

Among the influenza viruses, the HONi NWS (10) and WSN (1) mutants of A/WS (H_oN_1) are unique because they have encephalitogenic properties. We performed experiments to investigate the rate of covariation between the ability to reproduce at an increased temperature and the neurotropic properties of the NWS virus. Previous experiments failed to demonstrate a linkage between the two characters (2, 4).

We chose the NWS-D virus, selected after repeated passages in human diploid cells (5), for our experiments. This virus does not reproduce well at 39 C (12). To select a thermoresistant mutant of the NWS-D virus, repeated passages at progressively increasing temperatures (37.5 to 39 C) in HEF cells (a continuous cell line derived from hamster embryo fibroblasts) were performed. After 10 passages, virus, denoted NWS-tr, was isolated that grew equally well at 35 and 39 C and produced plaques with the same efficiency at both temperatures. This virus was plaque purified three times in HEF cells. We tried to induce ts mutants by incorporating 5-fluorouracil (0.9 to 0.09 μmol) into the medium of HEF cells infected with NWS-tr virus at a multiplicity of infection of 0.01 per cell. From 234 plaques formed by the progeny virus, virus lines were derived and examined for reproducing capacity at 39 C. The growth of one of these viruses was completely suppressed at this temperature. The virus, denoted NWS-ts, was plaque purified three times in HEF cells and was included in the subsequent experiments.

The neurotropic activity of the viruses was tested on white mice, strain H, weighing 7 to 8 g. They were inoculated intracerebrally with 0.03 ml of virus suspension, and the findings were evaluated as described previously (3). Ten mice were used for each dilution. The results of three independent tests with different virus stocks are summarized in Table 1. The tr virus was 100 to 2,500 times more neurovirulent in the different tests than was the original virus; on the other hand, the neurotropic activity of the ts mutant was markedly depressed (Table 1).

To examine whether mice not exhibiting the symptoms of encephalitis developed antibody against the virus, the surviving mice were bled on the 16th day after inoculation and the parallel sera were pooled and examined for the content of hemagglutination-inhibiting antibody (11). Antibody response was good in animals inoculated with larger virus doses; however, even among mice inoculated with small amounts of either virus, at least some also developed antibody (Table 2).

Our data indicate that the increased capability for growth at 39 C was linked with a marked increase in neurovirulence. On the other hand, acquisition of the ts character was associated with the loss of neurovirulence. The development of specific antibody in mice inoculated even with very small amounts of the ts mutant suggest that the loss of neurovirulence was not

due to the inability of this virus to replicate in mice.

TABLE 1. *Neurotropic activity of NWS-D virus and its derivatives*

Virus	Log_{10} difference between chicken embryo ID_{50} and mouse LD_{50}[a]		
	Expt 1	Expt 2	Expt 3
NWS-D	4.0	2.3	3.9
NWS-tr	1.0	0.2	0.5
NWS-ts	>4.0	3.9	4.6

[a] ID_{50}, Mean infective dose; LD_{50}, mean lethal dose.

TABLE 2. *Hemagglutination-inhibition (HI) antibody response in mice inoculated intracerebrally with different doses of NWS-D or NWS-ts virus*

Virus	Virus dose administered (log_{10} EID_{50}/mouse)[a]	Reciprocal of HI antibody titer[b]
NWS-D	5.0	160
	4.0	320
	3.0	160
	2.0	160
	1.0	<10
NWS-ts	4.0	320
	3.0	160
	2.0	40
	1.0	10
	0.0	10

[a] EID_{50}, Mean egg infective dose.

[b] Antibody titer in pooled sera of surviving mice (three to five animals per group).

LITERATURE CITED

1. Francis, T., Jr., and A. E. Moore. 1940. A study of the neurotropic tendency in strains of the virus of epidemic influenza. J. Exp. Med. **72**:717-728.
2. Hobson, D., E. A. Gould, H. I. Flockton, and M. G. Gregory. 1968. Laboratory differences between the WS strain of influenza A virus and its neurotropic variants. Brit. J. Exp. Pathol. **49**:516-524.
3. Janda, Z., and V. Vonka. 1968. Variation in neurovirulence of NWS influenza virus after repeated passages in different tissue culture systems. I. Derivation and neurotropic activity of NWS virus lines. Arch. Gesamte Virusforsch. **24**:192-196.
4. Janda, Z., and V. Vonka. 1968. Variation in neurovirulence of NWS influenza virus after repeated passages in different tissue culture systems. II. Correlation between neurovirulence and some other genetic markers. Arch. Gesamte Virusforsch. **24**:197-201.
5. Kutinová, L., V. Vonka, and D. Řezáčová. 1967. Plaque formation by NWS influenza virus in a human diploid cell strain. Acta Virol. **11**:372-378.
6. Maassab, H. F. 1969. Biologic and immunologic characteristics of cold-adapted influenza virus. J. Immunol. **102**:728-732.
7. Mackenzie, J. S. 1969. Virulence of temperature-sensitive mutants of influenza virus. Brit. Med. J. **3**:757-758.
8. Medvedeva, T. R., G. I. Alexandrova, and A. A. Smorodintsev. 1969. Pathogenicity of cryophilic and thermophilic strains of influenza type A2 virus for developing chick embryos and albino mice. Acta Virol. **13**:379-386.
9. Mills, J., R. M. Chanock, S. R. Nusinoff, B. E. Walls, and I. E. Fishburne. 1969. Temperature sensitive mutants of influenza virus. I. Behaviour in tissue culture and in experimental animals. J. Infect. Dis. **123**:145-157.
10. Stuart-Harris, C. H. 1939. A neurotropic strain of human influenza virus. Lancet **ii**:497-499.
11. Tučková, E., V. Vonka, and M. Stárek. 1968. Comparison of genetic properties of influenza virus before and after repeated passages in monkey kidney cell culture. Acta Virol. **12**:316-323.
12. Tučková, E., V. Vonka, and M. Stárek. 1971. Genetic interaction between two influenza A/WS virus mutants. I. Characterisation of the mutants. Acta Virol. **15**:337-344.