



Title	Isolation of a New Variant of HVJ Showing Low Cell Fusion Activity
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Isolation of a New Variant of HVJ* Showing Low Cell Fusion Activity

The Z strain of HVJ (Fukai and Suzuki,1955) has been continuously passaged weekly in embryonated eggs since its first isolation in 1953 in our laboratory.

One marked characteristic of the Z strain is its strong cell fusion activity (Okada *et al.*,1957; Okada,1958; Okada and Tadokoro,1961). Incidentally, the authors found that the cell fusion activity of an HVJ substrain passaged by Hosokawa, one of the authors, was lower than that of the parent strain. A comparison of the cell fusion activity of the Hosokawa and parental strains is shown in Table 1.

Table 1. Comparison of Fusion Activity of HVJ, Z Strain and a Substrain Passaged by Hosokawa

Virus	Virus titer used (HAU/ml)	Fusion Index
HVJ, Z (parent strain)	1,500	6.5
	3,000	6.6
	6,000	7.6
	12,000	8.2
Substrain passaged by Hosokawa	2,000	1.6
	4,000	2.6
	8,000	2.0
	16,000	1.7

For this determination, 0.5 ml of Ehrlich's ascites tumor cell suspension (1.2×10^7 /ml) and an equal volume of virus sample were mixed and reacted at 37°C for 60 minutes.

The determination of the fusion activity of the viruses was made with Ehrlich's ascites tumor cells. The degree of cell fusion activity is expressed as the fusion index (F.I.):

$F.I. = (\text{Cell number in control sample} / \text{Cell number in test sample}) - 1.0$ (Okada and Tadokoro,1961). The F.I. to virus ratio of the occurrence of the cell fusion varies remarkably between Hosokawa's strain and the parent, Z strain.

To obtain a pure clone, the virus sample was passaged at limiting dilution for five generations and the fusion activities of the samples from every generation were determined. Ten-fold serial dilutions of Hosokawa's strain (infected allantoic fluid) from 10^{-6} to 10^{-10} , were prepared in broth containing 1,000 U/ml of penicillin and 100 γ /ml of streptomycin, and an 0.2 ml aliquot of each dilution was inoculated into the allantoic cavity of five 10-day-old embryonated eggs. After 72 hours incubation at 35.5°C, the eggs were chilled and the chorioallantoic fluids

* Official name of the Sendai virus adopted by the Society of Japanese Virologists

were harvested. The most dilute sample showing virus infection was stored in a freezer as seed for the next generation and other infection fluids from lower dilutions were pooled and used for determination of the cell fusion activity after purification and concentration by differential centrifugation.

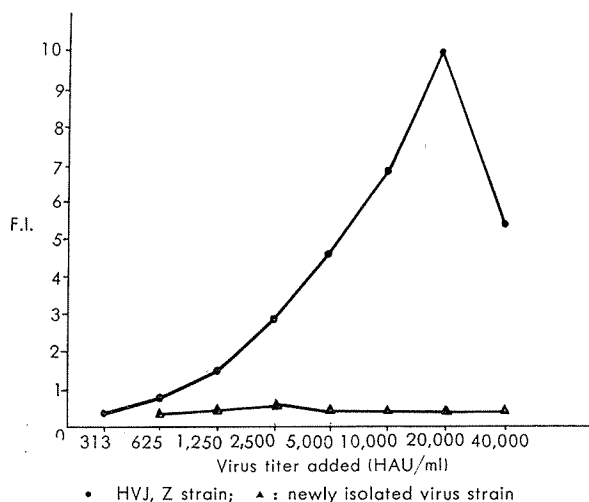


Fig. 1. Comparison of Cell Fusion Activity of Parental HVJ, Z Strain and Newly Isolated Virus Strain (prepared from the 1st Limiting Passage of Seed Virus)

For this determination, 0.5 ml of Ehrlich's ascites tumor cell suspension (1.2×10^7 /ml) and an equal volume of virus sample were mixed and incubated at 37°C for 60 minutes

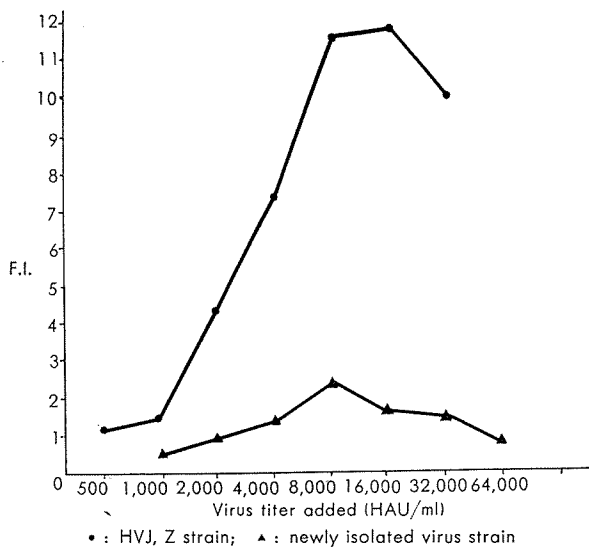


Fig. 2. Comparison of Cell Fusion Activity of Parental HVJ, Z Strain and Newly Isolated Virus Strain (Prepared from the 5th Limiting Passage of Seed Virus)

As indicated in Figs. 1 and 2, the parental HVJ,Z strain, showed a constant high fusion activity for five successive control passages while the activity of samples from the limiting dilution passages were much lower. The Infectivity/Hemagglutination ratios (I/A) of the two kinds of virus were nearly equal being about ca. $10^{6.0}$ and no noticeable difference was found in the virus yield and the growth rate in embryonated eggs. These facts indicate that the newly isolated strain is as well adapted to embryonated eggs as the old strain.

As the newly isolated strain also has hemolytic activity, it is not an Influenza virus. It is not a Mumps virus either because of the high yield and high growth rate of the strain in embryonated eggs. As shown in Table 2, the cross hemagglutination inhibition test between the old strain, the new strain and Newcastle disease virus, showed not only the antigenic coincidence between the old and new strains but also the difference between NDV and these two strains.

Table 2. Hemagglutination Inhibition Titer of Anti-HVJ and Anti-NDV Serum Against HVJ-Z, Newly Isolated Virus and Newcastle Disease Virus

Virus	anti-HVJ, Z serum	anti-NDV serum
HVJ, Z	1,600	<10
newly isolated virus	1,600	<10
NTV	<10	400

From the above data, the strain with a low cell fusion activity which was recently isolated from the Z strain of HVJ, is concluded to be a variant of the wild HVJ, Z strain. It is, however, not clear when and how this variant originated.

The fact that there are two different kinds of virus in HVJ as the cell fusion activity indicates, may be compared with similar findings in Herpes viurs reported by Gray *et al.* (1958), McNair Scott and McLeod (1959) and Nii and Kamahora (1961).

The authors named this newly isolated strain as HVJ, Hideko strain (HVJ,H strain). Hideko is the name of the mother of one of the authors. From the view point of their cell fusion activities, HVJ,Z strain was expressed as F⁺ and HVJ,H strain as F⁻.

Biophysical or other characteristics of the new strain will be studied further.

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