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DEVELOPMENT AND CHARACTERIZATION OF A LIVE VARICELLA VACCINE (OKA STRAIN)

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A live varicella vaccine (Oka strain) was developed by serial passage of the Oka strain isolated in our laboratory, in human embryonic lung cells (11 times at 34 C) and guinea pig embryo cells (12 times at 37 C). It is slightly temperature sensitive at 39 C and shows a higher ratio of infectivity in guinea pig embryo cells to infectivity in human embryo cells than wild-type strains. The DNA digest with Hpa I enzyme of the Oka strain contained one unique fragment (K), although its mobility differed only slightly from that of the corresponding fragment of wild-type strains. Studies with clinical varicella zoster virus (VZV) isolates from vaccinees indicated that tests on the ratio of infectivity in guinea pig embryo fibroblasts (GPEF) to that in human embryo fibroblasts (HuEF) and the profile of the DNA digest with Hpa I are useful for differentiation of the vaccine strain from wild-type strains.

The vaccine virus showed stable immunogenicity during at least 15 further repeated passages in human diploid cells, a character which seems helpful for production of a large quantity of vaccine virus for practical use.

DEVELOPMENT OF A LIVE VARICELLA VACCINE OF THE OKA STRAIN

The Oka strain of varicella-zoster virus (VZV) was isolated in primary human embryonic lung cells from the vesicles of a 3-year-old child with typical chickenpox. It was passaged 11 times in human embryonic lung cells at 34 C and 12 times in guinea pig embryo (GPE) cells at 37 C. The virus was then passaged in human diploid cells (WI-38 and MRC-5) several times to prepare an experimental vaccine (Takahashi et al., 1974, 1975).

TEMPERATURE SENSITIVITY TESTS ON THE VACCINE AND WILD-TYPE STRAINS

The infectivities of the vaccine strain and wild-type strains at high (39 C) and low (34 C) temperatures were compared. As shown in Table 1, the vaccine strain was slightly temperature sensitive at 39 C, unlike the wild-type strains. The foci of the vaccine strain were also smaller than those of wild-type strains at high temperature but similar in size to the latter at lower temperatures. Thus the vaccine strain was more temperature sensitive than the wild-type strains. However this difference did not seem large enough to be used routinely as a marker to differentiate the vaccine strain from wild-type strains (Takahashi et al., 1982;

TABLE 1. *Temperature-sensitivities of the vaccine and wild-type strains*

Strain	Passage number in HEL cells	Infectivity (PFU/0.2 ml)			Ratio 39 C/34 C
		39 C	37 C	34 C	
Oka (vaccine)		4.0×10^{1}	1.5×10^{3}	1.5×10^{3}	1/38
Oka (parental)	7	3.0×10^{2}	8.5×10^{2}	9.5×10^{2}	1/3.2
Izawa	7	7.0×10^{1}	4.0×10^{2}	4.0×10^{2}	1/5.7
Inoue	5	3.0×10^{2}	1.0×10^{3}	1.0×10^{3}	1/3.3
Kawaguchi	12	2.0×10^{2}	6.5×10^{2}	7.0×10^{2}	1/3.5

TABLE 2. *Infectivities of the vaccine (Oka) and wild-type strains of VZV in guinea pig embryo fibroblasts (GPEF) and human embryo fibroblasts (HuEF)*

Strain	Source	Passage number in HEL cells	Titer on GPEF cells (PFU/0.2 ml)[a]	Titer on HuEF cells (PFU/0.2 ml)[b]	Ratio a/b
Oka (vaccine)			7.0×10^{3}	8.5×10^{3}	0.82
Oka (parental)	varicella	10	2.8×10^{2}	6.8×10^{3}	0.041
Tsuchiyama	varicella	5	5.2×10^{1}	1.5×10^{3}	0.035
Inoue	varicella	7	6.6×10^{2}	1.3×10^{4}	0.051
Watanabe	varicella	6	5.8×10^{1}	1.6×10^{3}	0.036
Wada	varicella	6	3.8×10^{2}	6.2×10^{3}	0.061
Terada	varicella	9	2.2×10^{2}	1.7×10^{4}	0.031
Morita	zoster	2	1.2×10^{2}	6.5×10^{3}	0.018
Kato	zoster	4	3.8×10^{1}	1.2×19^{3}	0.032
Takenaka	zoster	4	1.5×10^{2}	7.0×10^{3}	0.021
Yamashita	zoster	5	5.8×10^{1}	1.3×10^{3}	0.045
Yamaguchi	zoster	7	1.2×10^{2}	1.9×10^{3}	0.063
Ellen	varicella		8.0×10^{1}	2.1×10^{3}	0.038
				Mean±SD	0.038±0.015

Hayakawa et al., 1984)

DIFFERENCE IN INFECTIVITIES ON GPEF AND HuEF CELLS OF THE VACCINE AND WILD-TYPE STRAINS

The infectivities of the vaccine strain and wild-type strains were assayed by plaque titration on GPEF cells and HuEF cells. As shown in Table 2, the vaccine strain showed a higher ratio of infectivity on GPEF to that on HuEF than any of the wild-type strains examined. This test is convenient and the results are reproducible. Therefore, it can be employed as a marker test on clinical isolates from vaccinees (Hayakawa et al., 1984).

CLEAVAGE PROFILES OF DNAs OF VACCINE AND WILD-TYPE STRAINS BY RESTRICTION ENDONUCLEASE

In DNA digests with restriction endonucleases, the Hpa I-K fragment of the parental and vaccine Oka strain differed in mobility from that of other wild-type strains, as shown in Figs. 1 and 2 (Hayakawa et al., 1984).

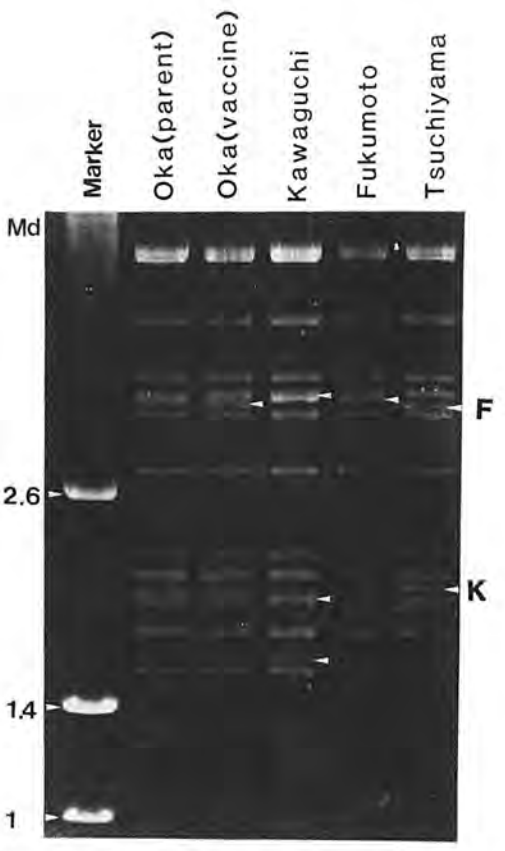


FIGURE 1. Cleavage profiles with restriction endonuclease (Hpa I) of DNAs of the parental Oka, vaccine Oka and two wild-type (Kawaguchi, Tsuchiyama) strains, and one strain recovered from a vaccine (FUKUMOTO). The variable segment produced by digestion with the restriction enzyme is indicated by an arrow. The segments formed are named alphabetically in order of decreasing size using those of vaccine strain as standards. Note fragment K.

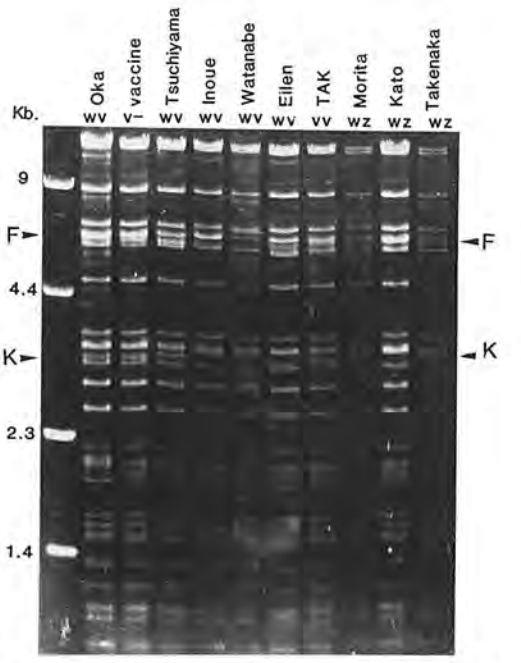


FIGURE 2. Cleavage profile with Hpa I enzyme of DNAs of seven wild-type strains (Tsuchiyama, Inoue, Watanabe, Ellen, Morita, Kato, Takenaka), the parental and vaccine Oka strains and one strain recovered from a vaccine (TAK). Note fragment K. The K fragment of parental and vaccine Oka strain and TAK strain show the same mobility, which differ from those of seven wild-type strains.

LABORATORY MARKER TESTS OF VZV CLINICAL ISOLATES FROM VESICLES OF VACCINEES

Nine clinical isolates from vesicles of vaccinees shortly or some time after vaccination were investigated by the above biological and biophysical marker tests. Details of the sources of the clinical isolates are given in Table 3.

In the test of the ratio of infectivity in GPEF to that in HuEF, the vaccine strain and representative wild strains were always included as controls and run in parallel with the clinical isolates to be tested. Representative results are shown in Table 3. All the strains except the KIT strain showed a high ratio of infectivity like the vaccine strain. One strain (OYA) showed a rather low ratio from this value, but it could not statistically ($p<0.01$) be regarded as a wild-type strain. The ratio for the KIT strain was below 0.1, indicating statistically ($p<0.05$) that it could be regarded as wild-type. In the cleavage profiles with Hpa I enzyme, the Hpa I-K fragment of all strains except the KIT strain differed in mobility from that of the Kawaguchi (wild-type)

TABLE 3. *Results of biological and biophysical marker test on the clinical VZV isolates from the vaccinees*

Case No.	Sex	*a*	Underlying diseases	Symptom	Period after vaccination	*b*	*c*	Clinical judgement	Infectivity ratio in GPEF/HuEF	Judgement	DNA pattern
1 (KIT)	M	4	ALL[d]	varicella	8 mo	3	+	wild	0.097	wild ($p<0.05$)	wild
2 (MEV)[e]	M	4	ALL	varicella	27 days	6	−	vaccine	0.65	vaccine	vaccine
3 (MEZ)[e]	M	4	ALL	zoster	12 mo	5	−	vaccine	0.76	vaccine	vaccine
4 (TAK)	M	9	ML[f]	varicella	27 days	3	−	vaccine	0.67	vaccine	vaccine
5 (ABE)	M	2	ALL	varicella	18 days	2	+	?	0.32	vaccine	vaccine
6 (OSA)	M	6	ML	varicella	15 days	7	+	?	0.81	vaccine	vaccine
7 (FMO)	F	7	ML	varicella	20 days	5	+	?	0.45	vaccine	vaccine
8 (KAK)	F	5	ML	varicella	11 days	3	+	?	0.36	vaccine	vaccine
9 (OYA)[g]	M	3	ALL	zoster	4 mo	2	−	vaccine	0.21	vaccine ($p<0.01$)	vaccine
Oka (vaccine)									0.59		vaccine
Kawaguchi						12			0.014		wild
Ellen									0.039		wild

Note: All strains were obtained from vesicles.
a Ages at the time of vaccination.
b Passage number of VZV in HEL cells.
c Contact with varicella patients.
[d] Acute lymphocytic leukemia.
[e] The MEV and MEZ strains were recovered from the same patient.
[f] Malignant lymphoma.
[g] Case 9 (OYA) showed mild symptoms of varicella 20 days after vaccination.

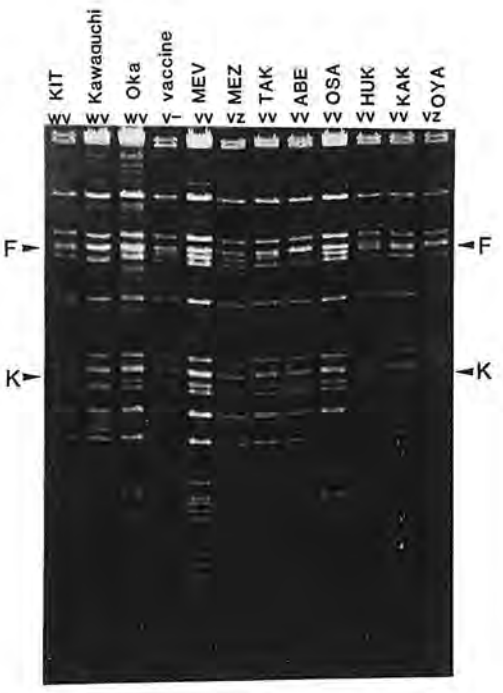
strain (Fig. 3).

The KIT strain was isolated from vesicles that developed in a vaccine recipient eight months after vaccination. This patient had been in contact with a varicella patient before developing vesicles. His neutralizing antibody titer was 1: 8 one month after vaccination, and when he developed vesicles 8 months after vaccination, his neutralizing antibody titer was less than 1: 2. From these finding it is reasonable that the isolated KIT strain showed the characters of a wild-type strain in laboratory tests.

Four patients (MEZ, MEZ, TAK, OYA) had had no contact with varicella patients at about the time of vaccination. Therefore, the isolated viruses were considered to correspond to the vaccine strain, and the results of laboratory tests were consistent with clinical findings (Hayakawa et al., 1984).

COMPARISON OF SEROLOGICAL RESPONSES TO VARIOUS DOSES OF THE VACCINE AND THE IMMUNOGENIC STABILITY OF THE VACCINE VIRUS DURING FURTHER PASSAGES

The serological responses to various doses of a single batch (Lot 7900) of vaccine in normal children, children with underlying diseases (other than malignant diseases) and children with leukemia were compared. Furthermore, vaccines (Lot 7900-10 and 7900-15) were prepared from the vaccine (Lot 7900) by 10 and 15 further serial passages in MRC-5 cells and their immunogenicities were examined to determine the immunogenic stability of the vac-



cine virus during serial passage in human diploid cells.

As shown in Table 4, a serological response was observed in nearly all normal children when more than 300 PFU/dose was given. Similar results were obtained in children with acute leukemia and in those with underlying diseases. The antibody responses with 1,500–2,500 PFU were slightly higher than those with 300–600 PFU, but the difference was not marked.

The serological responses of normal children

◀

FIGURE 3. Cleavage profile with Hpa I enzyme of DNA of virus from vaccinees (KIT, MEV, MEZ, TAK, ABE, OSA, HUK, KAK, OYA) and from the parental and vaccine Oka strains and a wild-type (Kawaguchi) strain. WV, Wild-type virus isolated a varicella patients; V-, Vaccine virus; VV, Vaccine virus isolated from vaccinees; WZ, Wild-type virus isolated from a zoster patient; WV′, Wild-type virus isolated from a vaccinee.

TABLE 4. *Comparison of serological responses of children vaccinated with various doses of virus after various numbers of passages in human diploid cells (MRC-5 cells)*

Lot No.	Viral dose (PFU)	Number vaccinated	Number seroconverted	Mean antibody titer (IAHA)
Children with acute leukemia				
7900	500	11	10 (91%)	$2^{3.9}$
	600	29	27 (93.1%)	$2^{4.1}$
Children with underlying diseases (other than malignancies)				
7900	300	12	12 (100%)	$2^{3.1}$
	590	28	27 (96.4%)	$2^{3.7}$
	600	95	91 (95.8%)	$2^{3.7}$
	1,500	11	11 (100%)	$2^{4.9}$
	2,500	11	11 (100%)	$2^{4.5}$
Normal children				
7900	300	122	119 (97.5%)	$2^{3.7}$
	500	56	52 (92.8%)	$2^{3.5}$
	600	59	57 (96.6%)	$2^{3.6}$
	1,500	42	42 (100%)	$2^{4.4}$
Normal children				
7900-10	500	14	14 (100%)	$2^{4.1}$
	750	5	5 (100%)	$2^{4.0}$
	1,000	17	17 (100%)	$2^{4.5}$
	1,500	21	21 (100%)	$2^{4.0}$
7900-15	500	25	25 (100%)	$2^{4.5}$

to 7900-10 and 7900-15 were similar to those to Lot 7900.

These results suggest that viral doses of 300–500 PFU are enough to immunize children including those with a high risk, and that the immunogenicity of the vaccine virus is stable during at least 15 further serial passages in MRC-5 cells. This fact should be helpful for production of a large quantity of vaccine for practical use.

ACKNOWLEDGMENT

Tables 1, 2, 3, Figs. 1, 2, 3 were reprinted from Hayakawa et al. J. Infect. Dis. 149: 956-963, 1984, by permission of the Univ. of Chicago Press (Copyright by the University of Chicago).

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