Looking Back on The Way to Develop a Live Vaccine Against Chickenpox and to Prevent Herpes Zoster: Current Status and Future Prospect

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I. BACKGROUND OF DEVELOPMENT OF OKA STRAIN OF LIVE VARICELLA VACCINE

1. ATTENUATION OF MEASLES VIRUS AND POLIO VIRUS

In 1959-1962, I worked on development of attenuated live measles vaccine in the laboratory of Professor Yoshiomi Okuno, Research Institute for Microbial Diseases Osaka University. This Institute was established in 1934, being full of refreshed activities of young researchers, yielding world remarkable findings such as oxygenase by Dr. Osamu Hayaishi in Department of Biochemistry and Cell fusion by Sendai Virus (Hemagglutination Virus of Japan, HVJ) by Dr. Yoshio Okada and others. Dr. Okuno had been in developing attenuated live measles by serial passage in the amniotic cavity and chorio-allantoic membrane of developing chick embryos. I had helped his studies. In addition, I was asked to do a study on adaptation of poliovirus type 1 and 3 to chick embryo cells with a hope to prepare live polio vaccine using chick embryo cells instead of monkey kidney cells. As well known at that time, poliovirus type 2 grows well in chick embryo cells, but type 1 and 3 do not. I attempted to adapt these viruses particularly type 3, which has been known unstable in human intestine, occasionally revert to virulent, developing paralysis of extremities, although rarely. The attempt finally failed; no continuous growth of poliovirus type 3 took place in chick embryo cells.

However, as few as after several alternate passages in chick embryo cells and monkey kidney cells, the virus was found to be thermosensitive (i.e., the titer of the passaged virus was lower at 39° than at 34°, whereas the titers of the original strain virus were comparable at these two temperatures) and to be remarkably less-neurovirulent when inoculated into thalamus of monkeys, being comparable with Sabins' attenuated Saukett's strain[1]) (Table 1a, 1b, 1c).

In brief, this observation confirmed that viruses become readily attenuated by passage in chick embryo cells less sensitive (foreign species cells) as compared with which was passaged in sensitive cells.

TABLE 1-A. Background of development of varicella vaccine (From Takahashi M, Hamada T, Okabe S. Biken J. 1963 Oct;6: 219-22; the author could not obtain permission because the Biken Journal office does not exist and one of the editors informed the author that there is no right holder.)

Attenuation of poliovirus

Passage of poliovirus (Type 3, Saukett strain) in developing chick embryo and alternate passages in monkey kidney cell and chick embryo cell cultures.

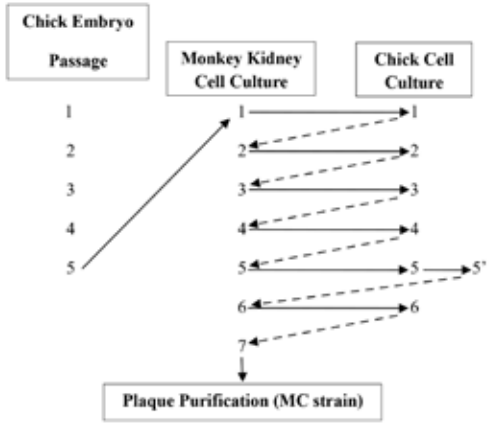

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TABLE 1-B. Comparison of original Saukett strain and its after alternate passage and a few (twice, 4 times, 6 times) passage in monkey kidney cell cultures and chick embryo cell culture (From Takahashi M, Hamada T, Okabe S. Biken J. 1963 Oct;6:219-22; see Table 1-a.) As attenuation passage proceed, infection of Saukett strain decreases at 40°C.

Virus	Virus Titer (PFU/0.2 mL)		Difference in Titer at 37 °C and 40 °C
	37 °C	40 °C	
Original Saukett Strain	$10^{6.8}$	$10^{6.8}$	0
CE_5 MK1	$10^{6.8}$	$10^{6.5}$	$10^{0.3}$
CE_5 $(MK\text{-}CC)_2$ MK	$10^{6.9}$	$10^{5.3}$	$10^{1.6}$
CE_5 $(MK\text{-}CC)_4$ MK	$10^{7.0}$	$10^{4.7}$	$10^{2.3}$
CE_5 $(MK\text{-}CC)_6$ MK	$10^{7.0}$	$10^{4.0}$	$10^{3.0}$
CE_5 $(MK\text{-}CC)_6$ MK +1 Plaquing (MC Strain)	$10^{6.9}$	$<10^{1}$	$>10^{5.9}$

TABLE 1-C. Neurovirulence test of original and its alternately passage of Saukett strain (twice, 6 times) in intrathalamic inoculation of monkeys (From Takahashi M, Hamada T, Okabe S. Biken J. 1963 Oct;6:219-22; see Table 1-a.) After 6 times alternate passage, poliovirus type 3 (Saukett strain) lost remarkably its neurovirulence.

Virus	Original Saukett Strain	CE5 (MK-CC)2 MK	CE5 (MK-CC)6 MK	CE5 (MK-CC)6 MK +1 Plaquing (MC Strain)
Viral dose (PFU/mL)	$10^{7.2}$	$10^{7.6}$	$10^{7.3}$	$10^{7.3}$
Paralysis	2/2	1/3	0/4	0/3

0.5 mL intrathalamic inoculation

This phenomenon was detected by Jenner around two centuries ago, but I confirmed that it really occurred after as few as several passages in foreign species cells, because poliovirus has distinct biological markers. I have been deeply impressed with these results, which was very useful for my later work on development of live varicella vaccine.[1]

2. MALIGNANT TRANSFORMATION OF CULTURED CELLS WITH HUMAN ADENOVIRUS AND HERPES SIMPLEX VIRUS

I had long been interested in the possible causative relationship of human viruses to human cancer. In 1962, tumor formation by adenovirus type 12 was reported in newborn hamsters.[2] Stimulated by that finding, and favored with good will of Rockfeller Foundation, I visited with my family Baylor Univ. College of Medicine in Houston, USA in 1963, where Dr. Trentin worked as founder of cancer formation in hamster with of adenovirus type 12. I started *in vitro* transformation experiments with adenovirus type 12; no viral growth or lytic viral infection was detectable in inoculated hamster embryo cells. In contrast, adenovirus type 5, which was classified as a nontumorigenic virus, cause lytic infection in hamster embryo cells. Both viruses are lytic to human embryo cells. Thus we tried to obtain conditional lethal mutants of adenovirus type 5[3,4] and to ascertain whether such mutants could – like adenovirus type 12- cause the transformation of hamster cells. We obtained both temperature-sensitive mutants, which grew at 38.5°C, and host-dependent mutants which caused lytic infection in human but not hamster embryo cells. Using an established hamster embryo cell line (Nil cells) that, unlike primary cultured cells, is readily transformed, we observed malignant transformation with both mutants.[4,5] However, we detected no transformation of human embryo cells with these mutants. This finding was consistent with the lack of evidence of a causative relationship of human adenoviruses with human cancer. Actually, Dr. Trentin had paid all efforts to obtain positive result in detecting adenovirus specific tumor antigen in dissected tumor tissue obtained from neighboring M.D. Anderson Hospital and Cancer Institute, but all his efforts failed. Thus, unfortunately, the causative relationship between human adenovirus and cancer became negative.

In 1971, Dr. Rapp[6] who was a good friend in the neighboring laboratory of virology at Houston reported that hamster embryo fibroblasts were transformed with ultraviolet-irradiated herpes simplex virus type 2 (HSV-2).[6] We found their work interesting and attempted to transform hamster cells with temperature-sensitive mutants at a nonpermissive temperature. Approximately 2,700 clones of HSV-2 from mutagenized stock virus and were isolated at 32°C, and 42 clones found to be nonpermissive at 38.5°C were examined for the ability to transform hamster and human embryo cells at 38.5°C. Hamster embryo ells were transformed by three mutants.[7] Transient transformation of human embryo fibroblasts was documented with one mutant, but resulted in the failure of serial passage of the

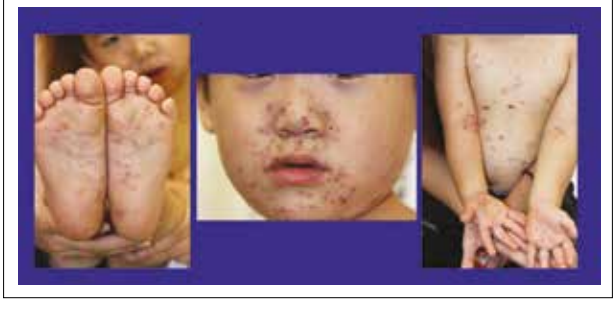

Fig 1. Typical rashes of varicellar (chickenpox). Various types of rashes appear simultaneously even in the palm and sole including severe scars.

cells so that the finding was not reproducible.[7] Later on, we attempted repeatedly to transform human embryo fibroblasts with ultraviolet-irradiated human HSV-2, but were unsuccessful.

Through these experiments, I became convinced that human adenovirus and HSV, although known to induce malignant transformation of hamster and rat embryo fibroblasts (i.e., foreign-species cells) are related little – if at all- to oncogenesis in human cells (i.e.,- indigenous cells).

3. MOTIVATIONS FOR AND PROBLEMS IN THE DEVELOPMENT OF A LIVE VARICELLA VACCINE

Chickenpox is usually a mild illness but occasionally manifests as a severe disease in children. (Fig 1) After a member of my family (3 years old son) had severe chickenpox in Houston in 1964, with high fever and widespread rashes lasting for 3 days, I began to consider how this disease might be prevented by vaccination. Since I knew that live vaccines induced solid immunity against diseases such as measles and polio, my thought from the beginning of the study was to develop a live attenuated varicella vaccine.

Two major problems had to be considered. The first was the possible oncogenicity of varicella-zoster virus (VZV), which is a herpes virus. Through the experiences described above, I had been convinced that HSV is either minimally or totally unrelated to malignancy in human cells. Although it was difficult to rule out VZV as a cause of malignancies, VZV had never been linked to any form of cancer. After my studies, Gelb et al.[8] reported that their fresh VZV isolates transformed hamster embryo cells morphologically, but they[9] later reported that this observation was not reproducible. Thus, even in vitro, it seemed unlikely that VZV could induce malignant change. The second problem was the possibility that live varicella vaccine virus would become latent, perhaps resulting in the later development of zoster. I presumed that attenuated virus would have less capacity than wild-type virus to replicate in humans and thus to become latent. In addition, I expected that symptoms of zoster caused by attenuated virus might be less severe than those of disease caused by wild-type viruses. Thus I thought that these two issues were not obstacles to the development of a live varicella vaccine.

4. DIFFICULTIES IN PREPARING "CELL-FREE" VZV

Since the earliest studies on in vitro propagation of VZV, it has been recognized that virus produced in cell cultures remains strongly cell associated; the inability to obtain cell-free infectious virus has hampered biological and immunological studies on this virus. Caunt[10], and Caunt and Taylor-Robinson[11] showed that infectious VZV could be isolated in a cell-free state following ultrasonic disruption of infected primary human thyroid cells. Shortly thereafter, Brunell[12] reported the isolation of cell-free virus from infected human embryo lung fibroblasts. Referring to those papers, we undertook studies to identify a suitable method for the isolation of cell-free virus from infected cultures and the composition of a suspending medium that would keep the infectivity of the virus as stable as possible. We reasoned that the following procedures would be likely to yield high-titered cell-free virus from infected cells: (1) use of cultured cells in the growth phase for inoculation of virus; (2) high-input multiplicity, with infected cells (rather than cell-free virus) used for inoculation because of the difficulty of obtaining a sufficient dose of cell-free virus; and (3) harvesting of the infected cell monolayer (by treatment with EDTA) before the appearance of advanced cytopathic changes with subsequent preparation of the infected cell suspension.[13]

Because VZV is highly heat-labile, particular caution was required in the selection of suspending medium that would preserve its infectivity.[11] After comparison of various media, simple phosphate buffered saline (C^{a++}, Mg^{++} free) was selected as the most suitable with sucrose (final concentration, 5%), sodium glutamate (0.1%), and fetal calf serum (10%, or 2.5% gelatin hydrolysate in case of vaccine preparation). With this medium, the decrease in infectivity during storage at -70°C was not detectable after storage for 1 year.

II. DEVELOPMENT OF OKA STRAIN LIVE VARICELLA VACCINE

1. PRIMARY ISOLATION OF VACCINE VIRUS

Fluid was taken from the vesicles of a 3-year-old boy who had typical chickenpox but was otherwise healthy. The fluid was stored at -70°C until it was inoculated onto primary cultures of human embryonic lung (HEL) cells. At a temperature of 34°C, characteristic foci appeared after 7-10 days. The virus was designated as the Oka strain since this was the surname of the boy from whose vesicular fluid was derived.[14]

2. RATIONAL FOR AND DESIGN OF A LIVE VARICELLA VACCINE

VZV spreads from cell to cell, forming distinct foci that are visible by microscopy even in unstained cell cultures and are clearly seen after methylene blue or fluorescent antibody staining. Cell-mediated immunity seems essential- or at least as important as humoral immunity – in preventing the spread of VZV in vivo. Since inactivated or subunit viral antigens are usually weak inducers of cell-mediated immunity, we reasoned that a live vaccine might be most useful for the prevention of varicella.

It had been very difficult to demonstrate the pathogenicity of VZV in experimental animals. Therefore, we anticipated that the attenuation of VZV would be proven only by extensive clinical trial, and that testing of only a limited number of candidate strains would be feasible. The classical (empirical) method of attenuation, as described previously, was used. Of the various kinds of nonprimate cultured cells tested for susceptibility to infection with the Oka strain of VZV, only guinea pig embryo fibroblasts (GPEF) were found susceptible. Of the several VZV strains tested, the Oka strain grew best in GPEF,[14,15] when initially used as the substrate cells for vaccine studies. Until the early 1970s, nonprimate primary cell cultures were preferred to continuous human cell lines for vaccine

production. In 1969, at a meeting on rubella vaccine at the U.S. National Institutes of Health[4], excellent data was presented by Plotkin et al.[16] concerning RA27/3 vaccine prepared in human diploid cells (WI-38). However, opposition to the diploid cell substrate was expressed by Sabin[17] who stressed the theoretical risk of human retrovirus in human diploid cells. On the basis of these objections, the first live rubella vaccines approved by U.S. federal authorities in 1969-1970 were prepared in duck embryo cell culture (HPV 77 strain)[18], dog kidney cells, and rabbit kidney cell culture (Cendehill strain)[19]. Soon thereafter the RA/27 human diploid fibroblast vaccine[20] was licensed in Europe.

On the basis of these results, we conducted further experiments aimed at the development of a live varicella vaccine. After the 11th passage of Oka-strain virus in HEL cells at 34°C, infected cells were trypsinized and inoculated onto GPEF. Characteristic CPE appeared in a few days, and the transfer of infected cells was repeated. Cell-free virus (1,000 to 2,000 PFU/mL) was extracted from infected cells by sonication. Passaged virus was identified as VZV by hemotoxylin-eosin staining, fluorescent antibody staining, and the neutralization test using HEL cells. Oka-strain VZV thus passaged 11 times in HEL cells and 6 times in GPEF was slightly more thermosensitive at 39°C than wild-type viruses and exhibited a greater capacity for growth in GPEF than the original or other wild-type strains. The biological and biophysical properties of this vaccine virus were described in detail in later reports[14,15]. Safety testing of the vaccine revealed a lack of pathogenicity (including intracerebral effects) in small nonprimate mammals and monkeys. The absence of C-type particles (suspicious leukemic virus) and of latent viruses was also confirmed morphologically and biochemically.

3. EARLY CLINICAL TRIALS: VACCINATION OF HEALTHY AND HOSPITALIZED CHILDREN

With the informed consent of their parents, healthy children who were living at home and had no history of varicella received various doses of Oka-strain virus passaged six times in GPEF. A dose of 500 PFU elicited seroconversion in 19 of 20 children. Even at a dose of 200 PFU, an antibody response was detected in 11 of 12 children. No symptoms due to vaccination were detected in these children. In short, sixth-passage Oka-strain virus in GPEF was well tolerate and immunogenic.

The first clinical trial of the vaccine in hospitalized children was undertaken in an effort to terminate the spread of varicella among children with no history of the disease.[14] In the hospital where the trial was conducted, chickenpox had frequently spread in the children's ward with severe cases on some occasions. In this protocol, children with no history of varicella were vaccinated

TABLE 2. Clinical and serological responses in children in hospital given a live varicella vaccine immediately after a case of varicella occurred (From Takahashi M, Otsuka T, Okuno Y, et al. Lancet. 1974 Nov 30;2(7892):1288-90; with permission from the Lancet Publishing Group.)

Underlying disease	Steroid therapy	C.F. antibody titres					Fever			Rash
		0 wk	1 wk	2 wk	4 wk	10 wk	Onset (days)	Maximum temperature (°C)	Duration (days)	
Purpura	+	<4	N.T.	32	64	16	13	37.7	1	-
Myelitis	+	<4	N.T.	32	32	N.T.	N.T.	N.T.	N.T.	-
Hepatitis	+	<4	N.T.	N.T.	32	N.T.	N.T.	N.T.	N.T.	-
Enteritis	-	<4	N.T.	N.T.	32	N.T.	N.T.	N.T.	N.T.	-
Arthritis	+	<4	N.T.	32	32	8	N.T.	N.T.	N.T.	-
Nephritis	-	<4	N.T.	N.T.	16	N.T.	N.T.	N.T.	N.T.	-
Asthma	-	<4	N.T.	16	32	N.T.	N.T.	N.T.	N.T.	-
Enteritis	-	<4	N.T.	4	32	16	10	37.5	1	+
Hepatitis	+	<4	N.T.	16	128	32	14	37.5	1	-
P.M.	+	<4	N.T.	16	16	N.T.	14	37.5	1	-
P.M.	+	<4	N.T.	N.T.	8	N.T.	N.T.	N.T.	N.T.	-
Haemangioma	+	<4	N.T.	N.T.	32	N.T.	N.T.	N.T.	N.T.	-
VSD	-	<4	N.T.	64	64	32	N.T.	N.T.	N.T.	-
Hepatitis	+	<4	N.T.	N.T.	16	N.T.	N.T.	N.T.	N.T.	-
Nephrosis	+	<4	N.T.	N.T.	32	N.T.	N.T.	N.T.	N.T.	-
Nephrosis	+	<4	<4	16	64	32	N.T.	N.T.	N.T.	-
Nephritis	+	<4	<4	4	32	N.T.	11	37.5	1	-
Nephritis	-	<4	<4	N.T.	32	N.T.	N.T.	N.T.	N.T.	-
Nephritis	-	<4	N.T.	32	16	N.T.	11	38.2	1.5	+
Nephritis	-	<4	4	32		N.T.	N.T.	N.T.	N.T.	-
Nephritis	-	<4	N.T.	N.T.	16	N.T.	N.T.	N.T.	N.T.	-
Enteritis	-	<4	N.T.	N.T.	32	N.T.	N.T.	N.T.	N.T.	-
Nephritis	-	<4	<4	N.T.	32	N.T.	N.T.	N.T.	N.T.	-

P.M. = purulent meningitis, VSD = ventricular septal defect
N.T. = Not tested

immediately after the occurrence of a case of varicella. These children suffered from conditions including nephritic syndrome, nephritis, purulent meningitis, and hepatitis. (Table 2) Twelve children had been receiving corticosteroid therapy. An antibody response was documented in all of the vaccinated children; within 10-14 days after vaccination six children developed a mild fever, and two of the six developed a mild rash. It was uncertain whether these reactions were due to vaccination or to naturally acquired infection modified by vaccination. No other clinical reactions or abnormalities of the blood or the urine were detected. Thus on this ward, the spread of varicella infection was prevented except in one case: a child who was not vaccinated because his mother mistakenly believed that he already had varicella became severely ill. This study offered the first proof that the Oka vaccine was well tolerated by patients receiving immunosuppressive therapy and stirred hopes that this vaccine would prove practical for the prevention of varicella.

4. CLINICAL TRIALS WITH VACCINES PREPARED IN HUMAN DIPLOID CELLS

VZV yield from GPEF cells was considerably lower than that from human embryo fibroblasts. In addition, the level of viral infectivity was found to decrease to approximately one-third of the original level during lyophilization. Thus, cells that would yield more virus were sought. Because the human diploid cell line WI-38 had been widely used for vaccine production, we decided to cultivate the Oka strain of VZV in WI-38 cells which was outgoing from Dr. Hayflick.[22] After 12 passages in GPEF, the virus was passaged several times in WI-38 cells. The virus thus obtained was subjected to the same safety testing described previously and was evaluated in clinical trials. When a shortage in the supply of WI-38 cells became a concern, MRC-5 cells which were kindly offered from Dr. J. Jacob[23] and Dr. Perkins (National Institute for Medical Research, U.K.) at an early passage level. I was deeply grateful to both of them for their special courtesy given to me. A master seed lot was prepared at the second passage level in MRC-5 cells after three passages in WI-38 cells, and vaccines were subsequently produced exclusively in MRC-5 cell.

In an examination of its protective efficacy, the resulting vaccine was given to susceptible household contacts immediately after exposure to varicella.[24] Twenty-six contacts (all children) from 21 families were vaccinated, mostly within three days after exposure to the index cases. None of the vaccinated children developed symptoms of varicella. In contrast, all 19 unvaccinated contacts (from 15 families), exhibited typical varicella symptoms 10-20 days after the onset of the index cases. In three families where one sibling contact received vaccine and the other did not, none of the vaccinated children developed symptoms, whereas all unvaccinated controls exhibited typical symptoms. In general, the antibody titers after clinical varicella were 8-10 times higher than those after immunization. This study clearly demonstrated that vaccination soon after exposure was protective against clinical varicella.

TABLE 3. Relationship between severity of symptoms and age of children at first outbreak of varicella (from Baba K, Yabuuchi H, Okuni H, Takahashi M. Pediatrics. 1978 Apr;61(4):550-5; with permission from the American Academy of Pediatrics.)

Age (mo)	No. of Cases	Clinical Symptoms		
		Mild	Moderate	Severe
Unvaccinated				
1	4	4	0	0
2	4	1	1	2
4	1	1	0	0
5	3	0	1	2
6	4	1	1	2
7	6	0	4	2
8	5	1	3	1
9	1	1	0	0
10	2	0	0	2
11	3	0	0	3
12-24	10	3	5	2
Total	43	12	15	16
Vaccinated				
11	2	0	0	0
12-27	31	8	0	0
Total	33	8	0	0

In another clinical study, immunized children on a hospital ward protected despite subsequent exposures to natural varicella and herpes zoster during the nine months after vaccination.[24] After two years of follow-up of 179 vaccinated children including 54 children who had been receiving steroid therapy, 50 (98%) remained seropositive in the neutralization test, and only one of 13 household contacts of cases manifested mild varicella (10 vesicles but no fever).[25] In an institution for children less than two years old, prompt vaccination had a similar protective effect. Varicella developed in an 11-month-old infant on a ward for 76 children.[26] A total of 33 children over 11 months of age were vaccinated; 43 children less than 11 months of age were not vaccinated, partly because they were expected to still possess maternal antibody. A small viral dose (80 PFU) was used for immunization. Of the vaccinated group, 8 developed a mild rash and 1 of these 8 had a mild fever (less than 38°C) in 2-4 weeks after vaccination. In contrast, typical varicella developed in all 43 unvaccinated children during the 10 weeks after onset of the index case. Symptoms were severe in 16 cases, with confluent vesicles and high fever; after recovery, scars remained in 13 of these 16 cases. These results suggested that vaccination with as little as 80 PFU frequently stopped the spread of varicella among children in close contact with one another.[26] (Table 3)

5. VACCINATION OF CHILDREN WITH MALIGNANT DISEASES

In the first vaccination trials in children with malignant diseases with virus doses of 200, 500, or 1,500 PFU, chemotherapy was suspended for 1week before and 1 week after vaccination. Of 12 immunized children with acute lymphocytic leukemia (ALL), 10 who had been in remission for 6 months or less, 1 for 9 months,

and 1 for 48 months, 4 had fewer than 3,000 white blood cells/mm, but most had positive skin-test reactions with dinitrochlorobenzene, purified protein derivative, or phytohemagglutinin. Three of the 12 children developed a mild rash, with 13 (with 1,500 PFU), 30, and 25 (with 200 PFU) papular or incomplete vesicles, respectively; one child had a fever (39oC) for 1 day about 3 weeks after vaccination.[27,28] These results offered hope that a live varicella vaccine could be administered, with some precautions, to high-risk children.

6. VIEWPOINTS REGARDING LIVE VARICELLA VACINE AFTER INITIAL CLINICAL TRIALS

The various viewpoints expressed regarding live varicella vaccine in 1977, after these early clinical trials, included a cogent commentary by Brunell[29] whose main points were as follows:

1. The vaccine itself may cause zoster; however, it will take decades to find out whether or not this is the case.

2. Unfortunately, markers predictive of the behavior of a given strain of VZV with respect to causing zoster have not been identified.

3. Immunity after vaccination may not be as long-lasting as that after natural infection; thus, vaccination may enhance the risk of the relatively severe disease that frequently follows in adulthood.

4. Since naturally occurring varicella can be severe or even fatal in immunocompromised children who are receiving steroids for various chronic conditions and in patients with leukemia, it is not clear whether a live varicella would protect these children or cause serious disease, and it will be hard to find out.

Albert Sabin[30] presented the following views on the matters discussed by Dr. Brunell:

1. There is a high probability that live varicella vaccine virus will cause zoster infrequently: the absence of lesions and clinical manifestations in vaccinated children indicates that there is only limited viral multiplications and dissemination in the body and thus the potential for only limited (or no) invasion of sensory ganglia.

2. The lack of markers for zoster is not a contraindication for the testing or use of live varicella vaccine; live measles and rubella vaccines are being used in the absence of disease-specific markers.

3. The duration of immunity following the injection of a varicella vaccine is, of course, important, but it can be determined.

Stanley Plotkin[31] expressed a viewpoint in opposition to Dr. Brunell's, emphasizing that authority, however well-meaning, should not stand in the way of gathering data as long as the consequences are weighed at each step. While stressing the need for caution, Brunell[32] replied that he wholeheartedly supported research that would increase the understanding of virus latency. Drs. C. Henry Kempe and Anne Gershon[33]) stated that although varicella vaccine might result in either an increase or a decrease in latency, there was a real possibility of the latter, and only long-term studies of vaccines would provide an answer. They reminded readers that in any experimental endeavor involving human beings, the risk/benefit ratio is of immense importance. On the basis of the available data, they concluded that the potential benefits of varicella vaccine might well outweigh the potential dangers, particularly in high-risk persons.

Thus, in 1977, conflicting opinions were exchanged among several distinguished scientists interested in viral vaccines. Most of them favored continued work on a live varicella vaccine, including further studies on the latency of vaccine virus and the likelihood of subsequent zoster.

7. CLINICAL VACCINE TRIALS IN THE UNITED STATES AND EUROPE

In February 1979, a workshop on VZV was held at the U.S. National Institutes of Health.[33] The main topic of discussion was whether or not varicella vaccine (Oka strain) should be evaluated in clinical trials involving high-risk children. Dr. Saul Krugman referred to good short-term results with the vaccine, which he thought deserved to be tested and supportive. An NIH Collaborative Study Group was organized (Chief, Dr. Anne Gershon of New York University and later of Columbia University), and clinical trials were started with Oka-strain (Oka-Merck Varicella Vaccine) live varicella vaccine produced by Merck Research Laboratories, West Point, Pennsylvania. Many excellent investigations were conducted by that group, including clinical reactogenicity, the frequency of household transmission from vaccinated acute leukemic children with rash, and the persistence of immunity.[34-37] Other study groups also conducted clinical trials, most of which yielded favorable results.[38-42] In Europe, clinical trials were conducted with Oka-strain varicella vaccine prepared by SmithKline RIT. In 1983, an Expert-Committee meeting (Chief, Dr. F.T. Perkins) was held at the World Health Organization in Geneva to prepare a manuscript entitled "Requirements for the Live Varicella Vaccine." The resulting document was circulated and reviewed by authorities around the world and finally was published in 1985.[43]

In the introduction of "Requirements for the Live Varicella Vaccine, it was written "Several vaccine strains of attenuated varicella virus have been developed and compared, and the Oka strain has been shown to have the most desirable attributes of low virulence which inducing adequate antibody response and protection against the disease. Those studies have included both normal and immunocompromised persons. The Oka strain was developed by Dr. M. Takahashi and colleagues at the Research Institute for Microbial Disease, Osaka University, Osaka Japan. These documents gave me deep inspiration and stirred up me for further progress. Meanwhile, in 1984, the Oka-strain live varicella vaccine produced by SmithKline RIT was licensed for administration to high-risk children in Austria, Belgium, Federal Republic of Germany, Ireland, Luxembourg, Portugal, Switzerland, and the U.K. In November 1984, a symposium on active immunization against varicella was held in Munich. The papers presented at this meeting were published in the following year.[44-56] I was personally encouraged by Dr. Plotkin's statement55 that, despite a few questions, varicella vaccine appears to have a bright future, and that the work of Professor M. Takahashi, conducted over

more than 10 years, deserved praise, as he has persevered in the face of criticism, bringing medical science to the point where we can contemplate the conquest of another widespread human disease.

Fortunately, when I started development of live varicella vaccine, a monograph on "Varicella Zoster Virus" was published by Talyor-Robinson and Caunt,[57] both researchers in UK, through Springer Verlag NEW YORK and Wien in 1972. In this book as many information as possible were collected and well arranged. Before clinical testing, I have read intensely and prepared for laboratory and clinical testing of candidate varicella vaccine, particularly in this monograph under subtitle of "Experimental Human Infection", results of several papers published in 1910-1930,[58,59] have shown results in inoculation of vesicular fluid collected from patients of varicella or herpes zoster to children. Those studies had been attracted my attention, which had done with the aim to prevent varicella infection in children. The results showed that in many cases "vaccination" was roughly successful as no or few infection could be observed in the inoculated children, although viral dose nor serological investigation were impossible and undeserved. However, having read this monograph gave me some relief on possible adverse clinical reaction of our candidate live varicella vaccine in clinical trial.

III. DETECTION OF VIREMIA BEFORE AND AT THE ONSET OF CHICKENPOX : HERPES ZOSTER AND VARICELLA VACCINE

It had been extremely difficult to isolate VZV from blood and secretions except in severely immunocompromised individuals. Moreover, no evidence of hematogenous spread of VZV in normal children had been documented. Nevertheless, our colleagues Ozaki et al. succeeded in isolating VZV at a high rate from the mononucleocytes of otherwise healthy children with chickenpox.[60-63] Within 5 hours of blood collection, these authors inoculated the mononucleocyte fraction onto human embryo cell cultures at a ratio of 1:1. When no cytopathic effect was observed, the cells were trypsinized and transferred onto a new monolayer. Virus was isolated from all of 4 children from whom blood samples were taken on day 1 of illness. It was also possible to isolate VZV during the incubation period (i.e., 1-5 days before the appearance of rash). In contrast, no VZV could be recovered from any of 28 children 4-14 days after vaccination (before the appearance of neutralizing antibody) even at a high dose of 5,000 PFU. These finding had significant implications for the pathogenesis of chickenpox and zoster and particularly for the issue of latency of vaccine virus in immunized individuals. (Table 4)

TABLE 4. Viral isolation from mononuclear cells and antibody responses after close contact with patients with varicella (modified from Asano Y, Itakura N, Hiroishi Y, et al. J Infect Dis. 1985 Nov;152(5):863-8; with permission from Oxford University Press.)

Day of testing after onset of varicella	Viral isolation from mononuclear cells	Detectable antibodies*
-11	0/3 (0)	ND
-7	0/4 (0)	ND
-6	0/1 (0)	0/1 (0)
-5	1/2 (50)	ND
-4	1/3 (33)	0/2 (0)
-3	ND	0/1 (0)
-2	4/4 (100)	0/4 (0)
-1	4/5 (80)	0/5 (0)
0	4/17 (24)	0/13 (0)
1	7/32 (22)	0/28 (0)
2	0/14 (0)	0/12 (0)
3	0/3 (0)	4/12 (33)
4	0/1 (0)	9/18 (50)
≥ 5	0/3 (0)	14/14 (100)

Data are no. of positive subjects/no. tested (%)
ND = not done
* Measured by the assay for fluorescent antibody to membrane antigen

The current views of the immunopathogenesis of chickenpox and VZV to viremia may be as follows: The initial site of infection with wild-type VZV may be the conjunctiva, the upper respiratory tract, or both. Conjunctival and respiratory infections have been demonstrated in a guinea pig model. The virus then replicates at a local site, probably in regional cervical lymph tissues. Lymph node infection by VZV has been confirmed by the detection of VZV antigen in lymph nodes at autopsy in a fatal zoster case. The incubation period of natural varicella infection is usually 14 or 15 days, and the duration of local viral replication is estimated to be 4-6 days. This estimate is justified by the effect of the immediate inoculation of household contact with live varicella vaccine. The vaccine virus bypasses the initial replication site of wild-type VZV, thereby inducing immunity so early as to prevent subsequent replication of wild-type virus.

Secondary viremia may follow after replication in some or all of the above-mentioned sites. Of greater magnitude than the primary viremia, secondary viremia delivers virus to the skin, thus causing the appearance of rash. The occurrence of secondary viremia in natural varicella infection has been demonstrated by the isolation of VZV from mononuclear cells of immunocompetent patients, as described above. In contrast, no VZV was recovered from vaccinees.

These results suggested that the replication of vaccine virus in susceptible viscera is of lesser magnitude than that of wild-type VZV but is sufficient to induce an immune response mainly in regional lymph nodes. Thus it seemed that viremia was proportional to the virulence of the virus involved and that it was correlated with the appearance of a rash in otherwise-healthy children.

It has generally been believed that VZV in the skin vesicles travels up the sensory nerves to posterior ganglia, where it persists; this seems to be the major route of virus migration. Hope-Simpson[64] noted that the pattern of incidence of zoster on individual sensory ganglia is similar to the distribution of the rash in chickenpox and may bear a direct relationship to it. This observation may explain why sensory ganglia, and not motor ganglia, are selected for viral lodgement. As mentioned at the beginning of this chapter, a major question about live varicella vaccine had

TABLE 5. Comparison of incidence of zoster in acute leukemic children with or without clinical reaction following vaccination (modified from Takahashi M, Baba K, Horiuchi K, et al. Adv Exp Med Biol. 1990;278:49-58; the author could not receive any response from Plenum Publishers despite several attempts to obtain permission.)

Years after vaccination	No. of cases that developed zoster		
	Rash (+) after vaccination	Rash (-) after vaccination	Total
-1	8	3	11
1-2	3	2	5
2-3	0	1	1
3-4	0	0	0
4-5	1	0	1
Total	12/70 (17.1%)	6/200 (2.3%)	18/330 (5.5%)
	17.1/2.3 = 7.4		
Total observation period (months)	3.217	10.894	
Cases per 100 person years	3.13	0.46	

been whether the vaccine virus becomes latent and causes the later development of zoster. Since zoster is relatively uncommon in healthy children, long-term follow-up of vaccinated healthy children was required to answer this question definitively. However, since children with acute leukemia tend to develop zoster soon after natural infection, it was assumed that careful observation of the incidence of zoster in vaccinated children with ALL would yield valuable insight. Thus, vaccinated leukemic children were followed closely for the development of zoster and compared with leukemic children who had had natural varicella.

In one study[65] group in Japan, the incidence of zoster among vaccinated and naturally infected acute leukemic children were 15.4% (n=52) and 17.5 % (n=43), respectively; in another,[66] the rates were 9.1% (n=44), and 21.6% (n=37), respectively. Clinical symptoms in vaccinated children were usually mild and untroublesome, while in the latter study group, one had moderate and the others had severe symptoms. Some VZV isolates from cases of zoster that developed in vaccinated patients with ALL were shown to be derived from vaccine virus. However, all of the individuals studied had underlying acute leukemia, and person-to-person variation in their physical condition might have complicated precise comparison of the incidence of zoster in the two groups. As I contemplated how we could obtain more definitive evidence on the incidence of zoster after vaccination of children with acute leukemia, it occurred to me that the incidence of zoster should be followed in two groups of children with acute leukemia---one group who developed rash after vaccination and one who did not. As noted previously, the major route by which VZV reaches ganglia seems to be along peripheral nerves from vesicles. If the incidence of zoster were found to be higher among children who developed a rash after vaccination than among those who did not, then we should be able to predict whether latent infection of vaccine virus will occur in immunized children depending on their reaction to the vaccine.

Thus, we made this comparison, and the results shed more light on the latency of vaccine virus in vaccine recipients.[67,68] In a retrospective follow-up study of children with acute leukemia, zoster occurred far more frequently in those who developed a rash after vaccination (17.1% or 3.13 cases per 100 person-years; n=70) than in those without rash (2.4%, or 0.46 cases per 100 persons-years; n=250). (Table 5) These figures suggested that an absence of rash after vaccination is directly correlated with a low incidence of zoster, which in turn indicates that the incidence of zoster is lower among vaccine recipients than among children who have natural varicella.[39]

In 1986, live varicella vaccine produced by the Research Foundation for Microbial Diseases of Osaka University was licensed in Japan for use in high risk children and for optional use in children at standard risk. In 1988, a live varicella vaccine of the Oka strain was licensed in Korea.

Studies from the United Stated have indicated more clearly that the incidence of zoster after vaccination of leukemic children is lower than that after natural infection. Brunell et al.[69] reported that 19 of 26 children with acute leukemia who had natural varicella developed zoster, while none of 48 vaccinees did. With adjustment for the duration of observation and exclusion of vaccinees who failed to have a sustained antibody response or to develop chickenpox, the risk of zoster was still lower among vaccinees (P=0.017). The investigators concluded that there is no reason to suspect that recipients of varicella vaccine are more likely to develop zoster than children who have varicella.

Further studies by the NIAID Collaborative Study Group showed clearly that absence of rash is correlated with incidence of zoster.[70] In their investigation of vaccinated children with acute leukemia who developed zoster, 11 had a rash due to VZV (a vaccine-associated rash in eight cases and breakthrough varicella in three). The two children in whom zoster developed without a VZV skin rash had zoster lesions at the site of vaccination. Of 268 vaccinated children with VZV rashes, 11 (4.1%) had zoster. In contrast, there were only two cases zoster (0.7%) among the 280 vaccinated children with no VZV rash (P= 0.02 by Chi-squared test with continuity correction). The relative risk of zoster in the children who had had a VZV rash was 5.75 (95% confidence interval, 1.3-25.7).

Besides the main migration route (i.e., via the sensory nerve), there may be a minor hematogenous route of migration by virus to the ganglia. As noted above, however, no viremia could be detected in healthy vaccine recipients. Therefore, whatever the route, it seems far less likely for the vaccine virus than for wild-type virus to become latent in the ganglia and cause subsequent zoster.

Given these results and current knowledge on the pathogenesis of herpes zoster, we can be convinced that immunization with live varicella vaccine would lead to significant decrease in incidence of herpes zoster.

Since live attenuated varicella vaccine was licensed in 1986 in Japan, several millions of children received varicella vaccine.

In 1995, Oka-strain live varicella vaccines (Pro-

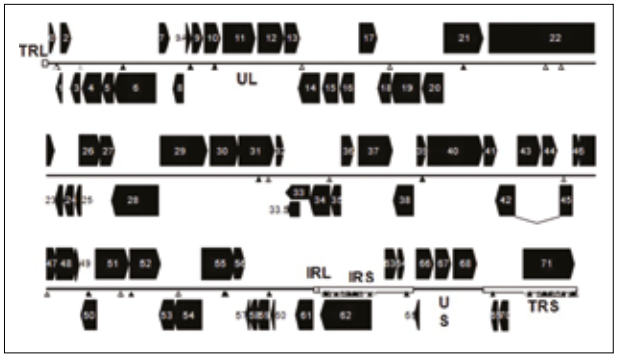

Fig 2. Mutations between the Oka vaccine and its parental viruses (From Y. Gomi. Advanced Applications for Vaccine Manufacturing. 2010 p.145; with permission from the CMC publishing Co. Ltd.)

duced by Merck Research Laboratories and SmithKline Beecham, respectively) were licensed for healthy children in the United States and Europe.

IV. COMPARISON OF THE COMPLETE DNA SEQUENCES OF OKA VARICELLA VACCINE AND ITS PARENTAL VIRUS

The DNA sequences of the Oka varicella vaccine virus (V-Oka) and its parental virus (P-Oka) were completed.[71,72] (Fig 2) Comparison of the sequences revealed 42 base substitutions, which led to 20 amino acid conversions and length differences in tandem repeat regions (R1, R3, and R4) and in an origin of DNA replication. Amino acid substitutions existed in open reading frames (ORFs) 6, 9A, 10, 21, 31, 39, 50, 52, 55, 59, 62, and 64. Of these, 15 base substitutions, leading to eight amino acid substitutions, were in the gene 62 region alone. Further DNA sequence analysis showed that these substitutions were specific for V-Oka and were not present in nine clinical isolates. The immediate-early gene 62 product (IE62) of P-Oka had stronger transactivational activity than the mutant IE62 contained in V-Oka in 293 and CV-1 cells. An infectious center assay of a plaque-purified clone (S7-01) from the V-Oka with 8 amino acid substitutions in ORF 62 showed smaller plaque formation and less-efficient virus-spreading activity than did P-Oka in human embryonic lung cells. Another clone (S-13) with only five substitutions in ORF 62 spread slightly faster than S7-01 but not as effectively as P-Oka. Moreover, transient luciferase assay in 293 cells showed that transactivational activities of IE62s of s7-01 and S7-13 were lower than that of P-Oka. Based on these results, it appears that amino acid substitutions in ORF 62 are responsible for virus growth and spreading from infected to uninfected cells. Furthermore, V-Oka virus was completely distinguishable from P-Oka and 54 isolates by seven restriction-enzyme fragment length polymorphisms that detected differences in the DNA sequence. Thus amino acid substitution in ORF62 seems to be related to the attenuation of Oka strain VZV.

V. ENHANCEMENT OF IMMUNITY AGAINST VZV BY GIVING VARICELLA VACCINE TO THE ELDERLY ASSESSED BY VZV SKIN TEST AND IAHA, GPELISA ANTIBODY ASSAY

The enhancement of immunity against VZV by subcutaneous injection of a live varicella vaccine was assessed by the VZV skin test for cell-mediated immunity (CMI), and immunoadherence hemagglutination assay (IAHA) and gpELISA antibody assays in the elderly people of 50 ~79 years of age. (Fig 3a,3b,3c,3d) A total of 127 subjects were examined: 79 aged 50~59, 25 aged 60~69, and 25 aged 70~79. All were seropositive by the gpELISA assay (1 was seronegative in the IAHA antibody assay).

In contrast, a notable decline was observed in the VZV skin test with increasing age. Negative reaction was observed in 16/79 (20.2%) of the subjects in their 50s, 12/25 (48.0%) in their 60s and 14/25 (56.0%) in the 70s. After the vaccination, the results of the VZV skin test changed from negative to positive in 15/16(91.8%) of subjects in their 50s, 11/12 (91.7%) in their 60s and 12/14 (85.7%) in their 70s. The mean antibody titer in the IAHA and the gpELISA increased approximately two fold after the vaccination in each group.

Immunity to VZV in 35 elderly subjects who were vaccinated previously was followed up for 4 years. All were positive by the VZV skin test after the previous vaccination. After 4 years, 31(88.6%) were positive by the skin test, 4 were negative and became positive after revaccination. Although this study was uncontrolled open study, the results suggest that administering live varicella vaccine to the elderly is effective for enhancing immunity, particularly CMI to VZV.

VI. PREVENTION OF HERPES ZOSTER AND POSTHERPETIC NEURALGIA IN OLDER ADULTS BY A CONCENTRATED VARICELLA VACCINE (OKA STRAIN, ZOSTER VACCINE)

The incidence and severity of herpes zoster and potherpetic neuralgia increase with age in association with a progressive decline in cell-mediated immunity to varicella-zoster virus (VZV). Oxman et al.[74] tested the hypothesis that vaccination against VZV would decrease the incidence, severity, or both of herpes zoster and postherpetic neuralgia among older adults as a Veterans Administration (VA) project, US.

They enrolled 38,546 adults 60 years of age or older in a randomized, double-blind, placebo-controlled trial of an investigational live attenuated Oka/Merck VZV vaccine ("zoster vaccine"). Herpes zoster was diagnosed according to clinical and laboratory criteria. The pain and discomfort associated with herpes zoster were measured repeatedly for six months. The primary end point was the burden of illness due to herpes zoster, a measure affected by the incidence, severity, and duration of the associated pain and discomfort. The secondary and point was the incidence of postherpetic neuralgia.

More than 95 percent of the subjects continued in the study to its completion, with a median of 3.12 years of surveillance for herpes zoster. A total of 957 confirmed cases of herpes zoster (315 among vaccine recipients and 642 among placebo recipients) and 107 cases of postherpetic neuralgia (27 among vaccine recipients and 80 among placebo recipients) were included in the efficacy analysis. The use of the zoster vaccine reduced the burden of illness due to herpes zoster by 61.1 percent ($P<0.001$),

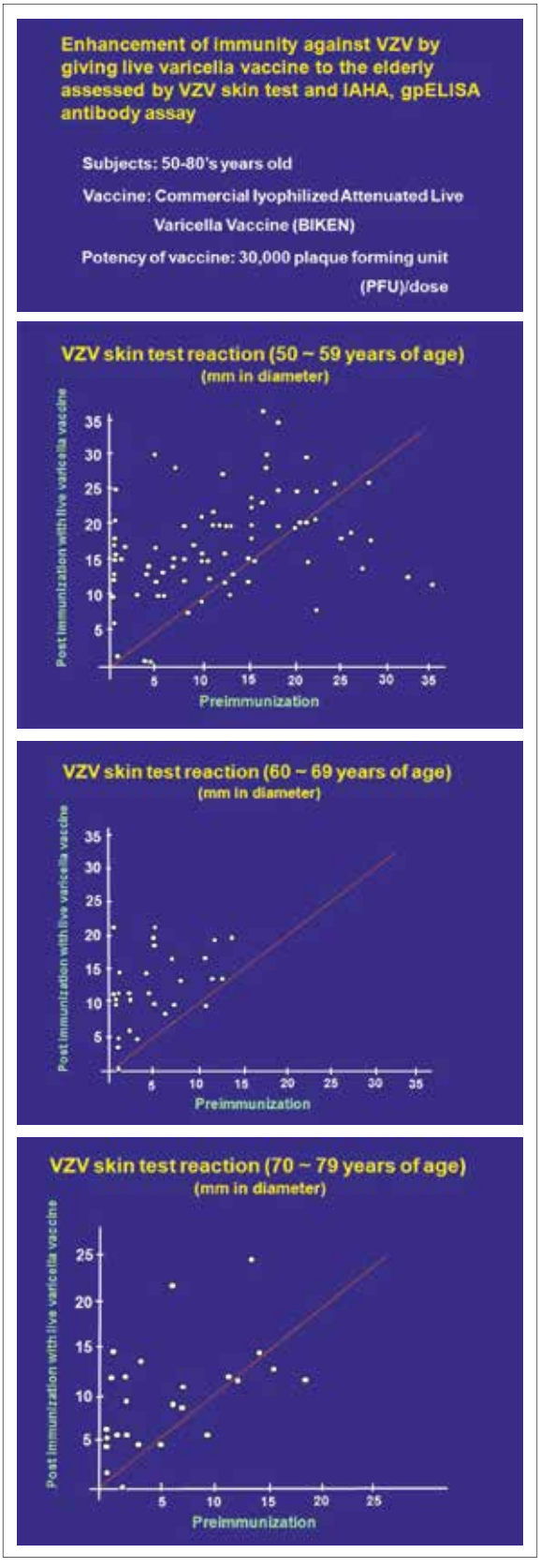

Fig 3. Enhancement of immunity against varicella-zoster virus (VZV) by giving attenuated live varicella vaccine (commercial lyophilized, potency of 30,000 plaque forming unit/dose; BIKEN) to the elderly, aged 50-80 years, assessed by VZV skin test and IAHA, gpELISA antibody assay. A) 50-59 years of age; B) 60-69 years of age; and C) 70-79 years of age (from Takahashi M et al. Vaccine. 2003 Sep 8;21(25-26):3845-53; with permission from ELSEVIER LTD.)[73]

and reduced the postherpetic neuralgia by 65.5 percent (P<0.001). Reactions at the injection site were more frequent among vaccine recipients but were generally mild.

FUTURE PROSPECT

It was recently reported that use of effectiveness of 2 doses of varicella vaccine (12-18 months old and 4~6 years old) is excellent, effectiveness of 2 doses of the vaccine was 98.3%.[74] Thus prevention of varicella has been improved. The author wishes that incidence of zoster would decline in the near future.

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