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Review

Development of a Live Varicella Vaccine–Past and Future

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SUMMARY: Background of the development of a live varicella vaccine, including studies on the attenuation of measles and polioviruses, and transformation experiments of cultured hamster and human cells with conditional lethal mutants of adenovirus and herpes simplex virus were described. Varicella-zoster virus (Oka strain) was passaged in guinea pig cells, and the resulting virus (vaccine virus) was found to have a higher affinity to guinea pig cells. It was recently proved that variations of base sequence occurred exclusively in gene 62 (immediate-early gene) in comparison of vaccine Oka virus and parent Oka virus. This variation is presumed to have occurred during passage in guinea pig cells. Live varicella vaccine (Oka strain) has increasingly been used throughout the world. It was also found in a preliminary study that giving the vaccine to the elderly enhanced humoral and cell-mediated immunity, leading to a prevention of post herpetic neuralgia. A large field trial is now going on in the United States to immunize the elderly for the purpose of prevention of herpes zoster, particularly post herpetic neuralgia.

Background

1. Attenuation of measles virus, rubella virus, and polioviruses

In 1959-1962, I worked on the development of attenuated live measles vaccines in the laboratory of Professor Yoshiomi Okuno, Osaka. This virus was attenuated by passage in the amniotic cavity and chorioallantoic membrane of developing chick embryos (1,2). Besides my work with measles virus, I was also asked to do a study on the adaptation of poliovirus types 1 and 3 to chick embryo cells. As is well known, poliovirus type 2 grew well in developing chick embryo cells, but types 1 and 3 did not. I attempted to adapt these viruses - particularly type 3 - to chick embryo cells by alternate passage in chick embryo cells and monkey kidney cells (3,4) (Table 1). The attempt finally failed: no continuous growth of poliovirus type 3 took place in chick embryo cells. However, after several alternate passages the virus was found to be thermosensitive (i.e., the titer of the passaged virus was lower at 34°C than at 39°C whereas titers of the original strain were comparable at these two temperatures) (Table 2) and to be less neurovirulent when inoculated into the thalamus of monkeys (Table 3).

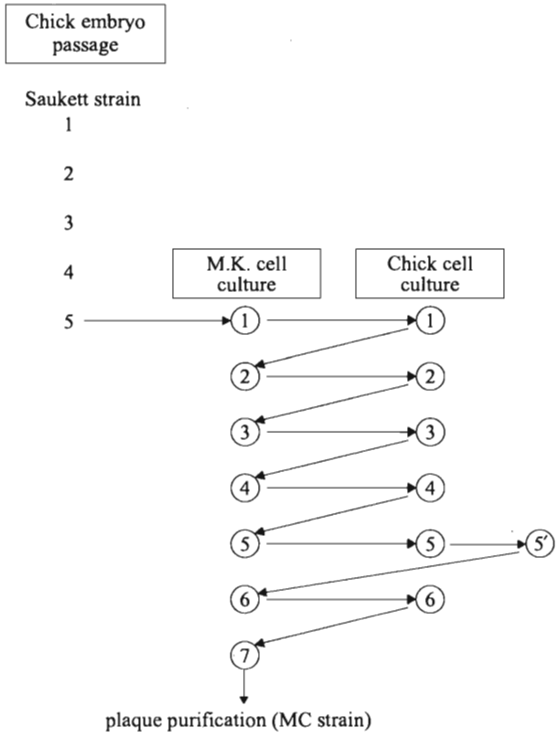
From these studies, I learned that passage in foreign-species cells is a convenient and effective means by which to attenuate viruses for use in live virus vaccines.

2. Malignant transformation of cultured cells with human adenovirus and herpes simplex virus

I had long been interested in the possible causative relationship between human viruses and human cancer. In 1962, tumor formation by adenovirus type 12 was reported in newborn hamsters (5). Stimulated by that finding, 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, caused 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 and to ascertain whether such mutants could - like adenovirus type 12 - cause the transformation of hamster cells. We obtained both temperature-sensitive mutants, which did not grow at 38.5°C, and host-dependent mutants, which

Table 1. Passage of poliovirus (Type 3, Saukett strain) in developing chick embryo and alternate passages in monkey kidney cell and chick embryo cell cultures



(from ref. 3)

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Table 2. Comparison of infectivity of original Saukett and alternately chick embryo cell-monkey kidney cell passaged viruses incubated at 37°C and 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
$CE_5 MK_1$	$10^{6.8}$	$10^{6.5}$	$10^{0.3}$
$CE_5 MK_3 CC_2$	$10^{6.9}$	$10^{5.3}$	$10^{1.6}$
$CE_5 MK_5 CC_4$	$10^{7.0}$	$10^{4.7}$	$10^{2.3}$
$CE_5 MK_7 CC_6$	$10^{7.0}$	$10^{4.0}$	$10^{3.0}$
$CE_5 MK_7 CC_6$ +1 plaquing (MC strain)	$10^{6.9}$	$<10^{1}$	$>10^{5.9}$

(from ref. 4)

Table 3. Comparison of neurovirulence of original and passaged poliovirus (Type 3, Saukett strain) in monkey by intracerebral inoculation

Virus	Original Saukett strain	$CE_5 MK_3 CC_2$[2)]	$CE_5 MK_7 CC_6$[3)]	$CE_5 MK_7 CC_6$ +1 plaquing
Viral dose (PFU/ml)[1)]	$10^{7.2}$	$10^{7.6}$	$10^{7.3}$	$10^{7.3}$
Paralysis	2/2	1/3	0/4	0/3

[1)]0.5 ml intrathalamic inoculation
[2)]Saukett strain. 5 chick embryo passage and 2 alternate in MK cell and chick cell and 1 MK cell passaged
[3)]Saukett strain. 5 chick embryo passage and 6 alternate passage in MK cell and chick cell and 1 MK cell passaged

(from ref. 3)

caused lytic infection in human but not hamster embryo cells (6). Using an established hamster embryo cell line (Nil cells) that, unlike primary cultured cells, was readily transformed, we observed malignant transformation with both mutants (7,8). 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 between human adenoviruses and human cancer.

In 1971, Duff and Rapp reported that hamster embryo fibroblasts were transformed with ultraviolet-irradiated herpes simplex virus type 2 (HSV-2) (9). 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 were isolated from mutagenized stock virus 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 cells were transformed by three mutants (10). Transient transformation of human embryo fibroblast was documented with one mutant, but resulted in the failure of serial passage of the cells so that the finding was not reproducible. 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 a malignant transformation of hamster and rat embryo fibroblasts (i.e., foreign-species cells), are little related - if at all - to oncogenesis in human cells (i.e., indigenous cells).

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. After a member of my family had severe chickenpox 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 member of the herpesvirus group. Through the experiences described above, I had become 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. (11) reported that their fresh VZV isolates transformed hamster embryo cells morphologically, but they later reported (12) 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.

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 immuno-

logical studies of this virus. Caunt (13) and Caunt and Taylor-Robinson (14) showed that infectious VZV could be isolated in a cell-free state following an ultrasonic disruption of infected primary human thyroid cells. Shortly thereafter, Brunell (15) 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 the virus; 2) high-input multiplicity, with infected cells (rather than cell-free virus) being 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.

Because VZV is highly heat-labile, particular caution was required in the selection of a suspending medium that would preserve its infectivity. After a comparison of various media, simple phosphate-buffered saline (Ca, 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)(16). With this medium, the decrease in infectivity during storage at –70°C was minimal; in fact, no decrease was detectable after storage for 1 year.

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 embryo 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 the vescular fluid it was derived (17).

Rationale 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 the 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 trials, 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 to be susceptible.

VZV (Oka strain) was passaged 11 times in HEL cells at 34°C and 12 times in GPEF cells at 37°C, and then propagated in human diploid cells (W1-38). The virus thus obtained exhibited an enhanced capacity for growth in GPEF than the original or other wild-strains. The biological and biophysical properties of this vaccine virus were described in detail in later reports (18-20) (Table 4,5). The results suggest that the vaccine virus is a variant with host-dependency.

Recently, variations between the vaccine virus and the parent Oka strain have been identified in nucleotides of viral gene 62 (immediate-early gene) (21). It is speculated that changes in gene 62, which seem to have occurred during passage in guinea-pig embryo cells, may be responsible for the attenuation of the parent Oka strain.

Early clinical trials: vaccination of healthy and hospitalized children

With the informed consent of the parents, healthy children

Table 4. Infectivity of vaccine (Oka) and wild-type strains of VZV in GPEF and HuEF

Strain	Source	No. of passages in HEL cells	Viral titer in indicated cells (PFU/0.2 ml)		Infectivity ratio (GPEF/HuEF)*
			GPEF	HuEF	
Oka					
Vaccine	—	—	7.0×10^{3}	8.5×10^{3}	0.82
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	8	3.8×10^{2}	6.2×10^{3}	0.061
Terada	Varicella	9	2.2×10^{2}	1.7×10^{4}	0.013
Morita	Zoster	2	1.2×10^{2}	6.5×10^{3}	0.018
Kato	Zoster	4	3.8×10^{1}	1.2×10^{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

GPEF; Guinea Pig Embryo Fibroblast, HuEF; Human Embryo Fibroblast
*The mean ratio (± SD) except for vaccine virus was 0.038±0.015

(from ref. 20)

Table 5. Adsorption of various strains of VZV to GPEL, GPES, and GPEK cells assessed by the infectious center assay

Virus	Input virus dose (PFU)	No. (PFU) of infectious centers (%) with		
		GPEL	GPES	GPEK
Oka (vaccine)	7.0×10^{2}	3.7×10^{2}(52.8)[a]	2.6×10^{2}(36.5)	1.3×10^{1}(1.8)
Oka (HEL, 5th passage)	4.8×10^{3}	1.9×10^{2}(4.0)	2.4×10^{2}(5.1)	9.6×10^{0}(0.2)
Kawaguchi (HEL, 10th passage)	1.1×10^{3}	8.7×10^{1}(7.9)	1.3×10^{2}(12.2)	5.5×10^{0}(0.5)
Inoue (HEL, 5th passage)	2.1×10^{3}	1.1×10^{2}(5.1)	8.8×10^{1}(4.2)	2.1×10^{0}(0.1)

GPEL; Guinea Pig Embryo Lung cells, GPES; Guinea Pig Embryo Skin cells
GPEK; Guinea Pig Embryo Kidney cells

a $\frac{\text{Number of infectious center at 4 h postinfection (PFU)}}{\text{Input virus (PFU)}} \times 100\%$

GPEL, GPES, and GPEK in 60-mm plastic plates were inoculated with the above dose of cell-free viruses. At 4 h after inoculation, cells were trypsinized, and an infectious center assay was performed on HuEF cells.

(from ref. 19)

who were living at home and had no history of varicella received various doses of Oka vaccine virus. 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.

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 (17). 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 immediately after the occurrence of a case of varicella. These children suffered from conditions including nephrotic syndrome, nephritis, purulent meningitis, and hepatitis. 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 vaccine, Oka strain was well tolerated by patients receiving immunosuppressive therapy and stirred hopes that this vaccine would prove practical for the prevention of varicella.

Clinical trials with vaccines prepared in human diploid cells

When a shortage in the supply of WI-38 cells became a concern (22), MRC-5 cells (23) were assessed. 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 cells.

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 3 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, one sibling contact received the 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.

In another clinical study (25), immunized children on a hospital ward were protected despite subsequent exposures to natural varicella and herpes zoster during the 9 months after vaccination. After 2 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) (26).

In an institution for children less than 2 years old, prompt vaccination had a similar protective effect (27). Varicella developed in an 11-month-old infant on a ward for 86 children. 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, eight developed a mild rash and one of these eight 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.

Vaccination of children with malignant diseases

In the first vaccination trials in children with malignant diseases with virus doses of 200, 500, or 1500 PFU, chemo-

therapy was suspended for 1 week before and 1 week after vaccination (18,28,29). Of 12 immunized children with acute lymphocytic leukemia (ALL), 10 had been in remission for 6 months or less, 1 for 9 months, and 1 for 48 months. Of these children, four had fewer than 3000 white blood cells/ mm^3, but most had positive skin-test reactions with dinitrochlorobenzene, purified protein derivative, or phytohemagglutinin.

Three of 12 children developed a mild rash; 13 (with 1500 PFU), 30 and 25 (with 200 PFU) papulae or incomplete vesicles; one child had a fever (39°C) for 1 day about 3 weeks after vaccination. These results offered hope that a live varicella vaccine could be administered with some precautions to high-risk children.

Most of the results of these clinical trials were reported in major journals of the United States (U.S.) and Europe, which drew strong attention of pediatricians, virologists, and those who were concerned with vaccine production.

Viewpoints regarding live varicella vaccine after initial clinical trials

Various viewpoints were expressed regarding live varicella vaccine. Philip Brunell (30) raised concerns about live varicella vaccine. His 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 (31) presented the following views on the matters discussed by 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 (32) expressed a viewpoint in opposition to Brunell's, emphasizing that authority, however wellmeaning, should not stand in the way of gathering data as long as the consequences weighed at each step. While stressing the need for caution, Brunell (33) replied that he wholeheartedly supported research that would increase the understanding of virus latency. C. Henry Kempe and Anne Gershon (34) 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 vaccinees 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 the vaccine virus and the likelihood of subsequent zoster.

Clinical vaccine trials in the U.S. and Europe

In the beginning of February 1979, a workshop on herpesviruses was held at the U.S. National Institute of Health (NIH) for 3 days. Approximately 200 experts of pediatric infectious diseases, virology, and public health section from the U.S., Canada and European countries attended the workshop. The main subject was whether or not varicella vaccine should be evaluated in clinical trials involving high-risk children. I was invited, being asked to talk on the details of results of vaccine development and its clinical trials in Japan. The last section of the workshop was general discussion. Saul Krugman first referred to good short-term results with the vaccine, which he thought deserved to be tested. There was no objection to his view. After the workshop, an NIH Collaborative Study Group was organized (Chief, Anne Gershon of New York University and later of Columbia University), and clinical trials were started with live varicella vaccine (Oka strain) produced by Merck Research Laboratories, West Point, Pa., USA. Many excellent investigations (35-38) 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. Other study groups also conducted clinical trials, most of which yielded favorable results (39-43). In Europe, clinical trials were conducted with varicella vaccine (Oka strain) prepared by SmithKline RIT, Rixensart, Belgium. In 1983, the expert committee (Chief, 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 was finally published in 1985 (44). Meanwhile, in 1984, the live varicella vaccine (Oka strain) produced by SmithKline RIT was licensed for administration to high-risk children in Austria, Belgium, the Federal Republic of Germany, Ireland, Luxenburg, Portugal, Switzerland and the United Kingdom. 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 (45). I was personally encouraged to read in this publication, Plotkin's statement (46) 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.

Herpes zoster and a live varicella vaccine

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 (47) 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 lodgment. As mentioned at the beginning of this review article, a major question about live varicella vaccine had been whether the vaccine virus becomes latent, resulting in 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 definitely. However, children with acute leukemia tend to develop zoster soon after natural infection. Therefore, it was assumed that careful observation of the incidence of zoster in vaccinated children with ALL would yield valuable insight. Vaccinated leukemic children were followed closely for the development of zoster and compared with leukemic children who had had natural varicella.

In one study group in Japan, the incidence of zoster among vaccinated and naturally infected children was 15.4% (n=52) and 17.5% (n=43), respectively (48); in another, the rates were 9.1% (n=44), and 21.6% (n=37), respectively (49). Clinical symptoms in vaccinated children were usually mild and untroublesome, while in the naturally infected children in the latter study group, one had moderate and the other had severe symptoms. Some VZV isolates from cases of zoster that developed in vaccinated patients with ALL were shown to be derived from the vaccine virus (20).

However, all of the individuals studied had underlying acute leukemia, and person-to-person variation in their physical condition might complicate precise comparisons of the incidences of zoster in the two groups. As I thought over 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 that developed rash after vaccination and one that 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 was 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 would occur in immunized children developing on their reaction to the vaccine.

Table 6. Comparison of incidence of zoster in acute leukemic children with or without clinical reaction following vaccination

Year after vaccination	No. of cases that developed zoster		Total
	Rash (+) after vaccination	Rash (–) after vaccination	
0-1	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%)	6/260 (2.3%)	18/330 (5.5%)
Total observation period (month)	3.217	10.894	
Cases per 100 person-years	3.13	0.46	

(from ref. 53)

Thus we made this comparison and the results shed more light on the latency of vaccine virus in vaccine recipients. In a retrospective follow-up study of children with acute leukemia, zoster occurred far more frequently in the group that developed a rash after vaccination (17.1% or 3.13 cases per 100 person -years; n=70) than in that without rash (2.4%, or 0.46 cases per 100 persons-years; n=250) (50-53) (Table 6). These figures suggested an absence of rash after vaccination is closely correlated with a low incidence of zoster, indicating that the incidence of zoster is lower among vaccine recipients than among children who had natural varicella.

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

Further clinical studies in the U.S. particularly on the incidence of zoster after vaccination

Studies from the U.S. have indicated more clearly that the incidence of zoster after vaccination of leukemic children is lower than that after natural infection. Brunell et al. (54) 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 the 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.

One comparative study included 84 matched pairs of U.S. children with underlying acute leukemia. Zoster developed in three (3.6%) of the 84 vaccinated subjects during 2.936 months of observation (an incidence of 1.23 cases per 100 person-years) and in 11 (13.1%) of the 84 naturally infected subjects during 4.245 months of observation: (an incidence of 3.11 cases per 100 person-years) (36).

Further studies by National Institute of Allergy and Infectious Diseases (NIAID) Collaborative Study Group elucidated clearly that an absence of rash is correlated with a low incidence of zoster (55). Of 268 vaccinated children with VZV rashes, 11 (4.1%) had zoster. In contrast, there were only two cases of zoster (0.7%) among the 280 vaccinated children with no VZV rash (P= 0.02 by χ^2 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 (56). However, no viremia could be detected in healthy vaccine recipients, while viremia could be detected in cases of natural varicella for several days before and just after appearance of the rash (57). 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 a significant decrease in the incidence of herpes zoster.

In 1995, live varicella vaccines (Oka strain) (produced by Merck Research Laboratories) were licensed for the universal

immunization of healthy children in the U.S.

Recent progress on varicella vaccine

Since the frequency and severity of herpes zoster increases with aging (58), and since VZV-specific T cell-mediated immunity (CMI) decreases with age, it was hypothesized that active immunization of older persons would boost their CMI against VZV and thereby decrease the frequency or severity (or both) of herpes zoster in vaccinees (59). Levin et al. (60) reported in their 6-year follow-up study of 130 vaccinated persons $\geqq$ 55 years of age, although the frequency of herpes-zoster like clinical events was within the expected range for this age cohort, the number of lesions was small, there was very little pain, and there was no post-herpetic neuralgia.

On the basis of their results, a large scale immunization trial for nearly 40,000 persons over 60 years of age, with half of them are placebo group, has been going on in the U.S. using high titered varicella vaccine by the support of NIH. The result will come out in 3-4 years.

Our recent study has demonstrated that the administration of booster doses of varicella vaccine to elderly individuals produces enhanced immunity to VZV, as indicated by levels of anti-VZV antibodies and skin reactivity to VZV antigen (61). We are hoping that live varicella vaccine would be of help for prevention of herpes zoster, particularly for post herpetic neuralgia.

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