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Complete DNA Sequences of Two Oka Strain Varicella-Zoster Virus Genomes▽

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Varicella-zoster virus (VZV) is a herpesvirus and is the causative agent of chicken pox (varicella) and shingles (herpes zoster). Active immunization against varicella became possible with the development of live attenuated varicella vaccine. The Oka vaccine strain was isolated in Japan from a child who had typical varicella, and it was then attenuated by serial passages in cell culture. Several manufacturers have obtained this attenuated Oka strain and, following additional passages, have developed their own vaccine strains. Notably, the vaccines Varilrix and Varivax are produced by GlaxoSmithKline Biologicals and Merck & Co., Inc., respectively. Both vaccines have been well studied in terms of safety and immunogenicity. In this study, we report the complete nucleotide sequence of the Varilrix (Oka-V_{GSK}) and Varivax (Oka-V_{Merck}) vaccine strain genomes. Their genomes are composed of 124,821 and 124,815 bp, respectively. Full genome annotations covering the features of Oka-derived vaccine genomes have been established for the first time. Sequence analysis indicates 36 nucleotide differences between the two vaccine strains throughout the entire genome, among which only 14 are involved in unique amino acid substitutions. These results demonstrate that, although Oka-V_{GSK} and Oka-V_{Merck} vaccine strains are not identical, they are very similar, which supports the clinical data showing that both vaccines are well tolerated and elicit strong immune responses against varicella.

Varicella-zoster virus (VZV) is a human alphaherpesvirus that causes chicken pox (varicella) and shingles (herpes zoster) (75). VZV has a linear, double-stranded DNA genome of approximately 125 kb that encodes at least 71 proteins (12). Primary infection with VZV results in varicella, which is a widespread, highly contagious disease. Varicella is commonly regarded as a mild childhood illness, but it may lead to serious complications, such as secondary bacterial infection, pneumonia, encephalitis, congenital infection, and death (76).

Like other herpesviruses, VZV has the capacity to persist in the body after the primary acute infection as a latent infection in sensory nerve ganglia. This lifelong latent infection commonly reactivates to cause herpes zoster, typically in elderly or immunocompromised patients (65).

In 1974, Takahashi et al. reported the development of a live-attenuated varicella vaccine through serial passages of wild-type virus in cell culture (67). The parental virus, Oka-P, was isolated in primary human embryo lung cell culture from vesicle fluid from a 3-year-old boy with typical varicella. The virus was attenuated by 10 passages in HEL and 12 passages in guinea pig embryo cells, plaque-purified, and passaged five times further in human diploid cells (WI38) to prepare a strain suitable for use as a vaccine (Oka-V) (Fig. 1) (67).

The Oka-V strain was first supplied in 1976 under license from the Biken Institute in Japan. Several manufacturers (SmithKline RIT, Merck Sharp & Dohme, and Pasteur Mérieux) subsequently used the Oka-V strain in the development of proprietary vaccines. A product license was obtained for Varilrix (frozen formulation) in 1984 by SmithKline RIT for use in groups at high risk for severe varicella and their healthy close contacts. SmithKline RIT—now GlaxoSmithKline (GSK) Biologicals—subsequently developed a refrigerator-stable formulation of this varicella vaccine. Varilrix is indicated in many countries for use in healthy and immunocompromised subjects from 9 months of age. GSK Biologicals' varicella vaccine production is based on the seed lot system (6, 14) using classical cell culture methods (Fig. 1). A manufacturer's working cell bank of human diploid cells, MRC-5, was prepared and tested according to World Health Organization requirements.

The Biken vaccine was licensed in Japan and Korea, in 1986 and 1988, respectively, for use in healthy subjects, and a license for Varivax with the same indication was granted in the United States in 1995 (1). In 1993, the vaccine manufactured by Pasteur Mérieux was licensed in France for use in potentially immunocompromised subjects.

Although the varicella vaccine is licensed in many countries, it is not routinely used because complications associated with varicella disease are often underestimated. Universal mass vaccination against varicella is implemented only in few countries; however, it is under consideration in many others (38, 40, 54, 72). The incidence of varicella disease and the rate of varicella-related hospitalizations in the United States have declined by about 80% since implementation of universal mass vaccination against varicella (using Varivax) in 1996 (8, 16, 81). A similar

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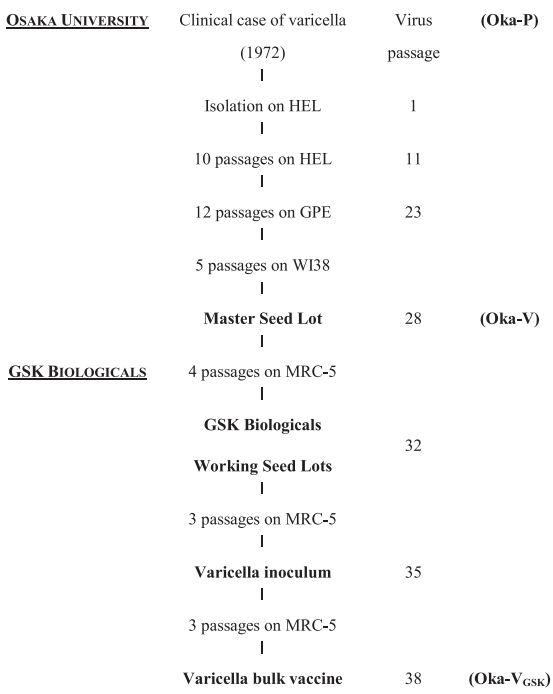

FIG. 1. Passage history of the live attenuated Oka varicella vaccine. HEL, human embryonic lung cells; GPE, guinea pig embryo cells; WI38 and MRC-5, human diploid cells.

decrease was observed in Uruguay since the introduction of varicella vaccination (using Varilrix) into the routine childhood immunization program in 1999, with the greatest reduction in children aged 1 to 4 years (51). Most pre- and postlicense studies showed that vaccination with one dose of varicella-containing vaccine provides 70% to 90% protection from chicken pox and over 95% protection against the most severe forms of the disease for a 7- to 10-year period after vaccination (2, 17, 33, 33a, 34, 40, 61). However, vaccine-induced immunity wanes over time (9), leading countries such as the United States to recommend a two-dose schedule for varicella vaccination (40). This strategy aims to overcome primary vaccine failures and to improve long-term protection, thereby reducing the risk of breakthrough varicella (4). Combined vaccine products containing the VZV Oka strain have been developed as well. For instance, GSK Biologicals and Merck & Co., Inc., developed combined tetravalent measles-mumps-rubella-varicella vaccines (Priorix-Tetra and ProQuad, respectively), providing the benefits of measles-mumps-rubella and varicella vaccination in a single injection (19, 30, 35, 48, 71, 72, 79).

Different sets of serological readouts have been used to characterize the adaptive humoral immune response after varicella vaccination or infection (4, 13, 26, 31, 34, 58, 73, 74). Comparative analysis has raised the possibility that differences in the genetic code between the vaccine strains could be responsible for disparity in vaccine-induced humoral responses (36).

Oka-V, and presumably its derivative vaccine strains, was not cloned during the development and the preparation of vaccine (67). Sequencing of the complete genome of the original Oka-V vaccine preparation revealed that it contained multiple variants that could be separated in cell culture (20, 22).

The aim of the present study was to analyze the complete consensus nucleotide sequences of Oka-V strain viruses contained in Varilrix (GSK Biologicals; Oka-V$_{GSK}$) and Varivax (Merck & Co., Inc.; Oka-V$_{Merck}$) and to compare them to the published sequences of Oka-V and Oka-P (22). The full-length genomic sequences were also compared to published partial sequencing information on Oka-V$_{GSK}$ and Oka-V$_{Merck}$ (3, 32, 60, 63).

MATERIALS AND METHODS

Nucleic acid extraction. Total DNA was extracted from a single vial of recent production lots of Varilrix (lot VAV10118, produced in April 2002; GSK Biologicals, Rixensart, Belgium) and Varivax (lot 0895 M, purchased in 2003; Merck & Co., Inc., Whitehouse Station, NJ) vaccines using a High Pure viral nucleic acid kit from Roche (Basel, Switzerland). In brief, 100 μl of sample was lysed in a lysing-binding buffer in the presence of proteinase K. The lysis mixture was then applied to a glass fiber filter, which binds the nucleic acids in the presence of the lysis and binding buffer containing chaotropic salts. Bound nucleic acids were eluted in 50 μl of nuclease-free water by centrifugation and stored at −70°C.

PCR. Around 540 primers were designed using Primer D software (GSK in-house software) and the nucleotide sequence of the Dumas strain (GenBank accession no. X04370) (12). Overlapping primers were designed approximately 500 bases apart to cover the entire genomic sequence of VZV. Sequences of primers used for amplification and sequencing are available upon request. The reaction mixtures for PCR contained 15 μl of HotStarTaq Plus Master Mix solution (Qiagen, Valencia, CA), 0.3 μM of each primer, and 5 ng of template DNA. A Tetrad thermal cycler (MJ Research, Waltham, MA) was used for all amplifications. An initial hot-start PCR step of 96°C for 15 min was followed by 35 cycles of amplification (95°C for 20 s, 55°C for 30 s, and 72°C for 45 s) and a final elongation step at 72°C for 3 min. All amplified products were then purified using QIAquick PCR purification kit (Qiagen). Direct sequencing of both DNA strands was performed on the generated amplicons.

Sequencing. Direct sequencing of purified PCR products and plasmid DNA was performed with BigDye Terminator cycle sequencing kit and a 3730XL genetic analyzer (both from Applied Biosystems, Foster City, CA). The viral sequences were compiled and analyzed with Sequencher software (Gene Codes Corp, Ann Arbor, MI). The following GenBank sequences were used for comparison: for the European (The Netherlands) reference strain (Dumas), X04370 (12); for Oka-P, AB097933 (22); and for Oka-V, AB097932 (22), AF206304 (3), AY016450 (15), and the sequencing information provided by Schmidt et al. (60). Unless otherwise stated, all described nucleotide sequence positions in this paper correspond to the genome of Dumas strain, X04370 (12).

Cloning of PCR products. When direct sequencing did not generate information of sufficient quality or when particular single nucleotide polymorphisms (SNPs) could not be reliably confirmed, additional subcloning was performed, followed by sequencing of numerous generated clones to confirm the consensus sequence of the region. Direct sequencing of the PCR products derived from regions with highly complex secondary structure (flanking regions between internal repeat long and internal repeat short regions, and the R3 repeat region) was complemented by subcloning of amplicons and sequencing of plasmid clones. PCR products containing these regions were individually inserted into a pCR2.1 vector (Invitrogen, Carlsbad, CA) and then transformed into competent *Escherichia coli* by the TOPO TA cloning method (Invitrogen). The plasmid DNAs were purified from cultured bacteria with a QIAprep spin kit (Qiagen). DNA sequences of the cloned inserts were determined using vector-specific sequencing primers.

Sequencing of ends of the viral genomes. The direct sequencing data for viral genome ends were complemented by sequencing of overlapping amplicons generated after circularization using T4 DNA ligase (Roche). The PCR mixtures contained 500 μM of each deoxynucleoside triphosphate, 10 pmol of each primer, and 2.5 U high-fidelity Platinum *Taq* polymerase (Invitrogen). PCR products were inserted into a pCR4-TOPO vector and transformed into competent *E. coli* TOP10 bacteria by the TOPO TA cloning method (Invitrogen). The plasmid DNAs were purified with a QIAprep spin kit (Qiagen). The consensus sequence of the cloned amplicons was confirmed by sequencing and alignment of multiple *E. coli* plasmid clones.

Nucleotide sequence accession numbers. The complete nucleotide consensus sequences of the Oka-V$_{GSK}$ (Varilrix) and Oka-V$_{Merck}$ (Varivax) strains are

TABLE 1. Complete Oka-V_{GSK} genome annotation

Start	Stop	Feature[a]	ORF	Function or comment
88	89	Miscellaneous		TRL/UL boundary
914	587	Gene	1	
592	587	Poly(A) signal		
914	588	CDS	1	
1133	1861	Gene	2	
1133	1849	CDS	2	
1856	1861	Poly(A) signal		
2446	1889	Gene	3	
1894	1889	Poly(A) signal		
2446	1907	CDS	3	
4140	2781	Gene	4	
2781	2776	Poly(A) signal		
4140	2782	CDS	4	Transactivator, tegument protein
5273	4251	Gene	5	
5273	4251	CDS	5	gK
8576	5325	Gene	6	
8606	9398	Gene	7	
9393	9398	Poly(A) signal		
8606	9385	CDS	7	
10666	9425	Gene	8	
9430	9425	Poly(A) signal		
10666	9476	CDS	8	Deoxyuridine triphosphatase
10641	10904	CDS	9A[b]	gN
11008	11963	Gene	9	
11958	11963	Poly(A) signal		
11008	11916	CDS	9	Syncytium formation, virion protein
12159	13420	Gene	10	
13415	13420	Poly(A) signal		
12159	13391	CDS	10	Transactivator, tegument protein
13589	16076	Gene	11	
13936	14196	Repeat region		Reiteration R1
16071	16076	Poly(A) signal		
13589	16003	CDS	11	
16168	18153	Gene	12	
18695	19350	Gene	13	
19345	19350	Poly(A) signal		
18395	19300	CDS	13	
21067	19296	Gene	14	
19301	19296	Poly(A) signal		
20526	20851	Repeat region		Reiteration R2
21067	19385	CDS	14	
22432	21198	Gene	15	
21203	21198	Poly(A) signal		
22432	21212	CDS	15	
23748	22522	Gene	16	
24103	25468	Gene	17	
25463	25468	Poly(A) signal		
24103	25467	CDS	17	
26444	25501	Gene	18	
25506	25501	Poly(A) signal		
26444	25524	CDS	18	Ribonucleotide reductase, small subunit
28796	26469	Gene	19	Ribonucleotide reductase, big subunit
30426	28956	Gene	20	
28961	28956	Poly(A) signal		
30426	28975	CDS	20	
30710	33856	Gene	21	
33851	33856	Poly(A) signal		
30710	33826	CDS	21	Nucleocapsid
34034	42341	Gene	22	
41405	41470	Repeat region		Reiteration R3
42336	42341	Poly(A) signal		
34034	42325	CDS	22	
43090	42378	Gene	23	
42383	42378	Poly(A) signal		
43090	42383	CDS	23	
43973	43163	Gene	24	
43168	43163	Poly(A) signal		
43973	43164	CDS	24	
44570	44083	Gene	25	
44088	44083	Poly(A) signal		
44570	44100	CDS	25	
44458	46125	Gene	26	
46079	47195	Gene	27	
47190	47195	Poly(A) signal		
46079	47080	CDS	27	
50588	46983	Gene	28	
46988	46983	Poly(A) signal		
50588	47004	CDS	28	DNA polymerase
50809	54460	Gene	29	
54455	54460	Poly(A) signal		
50809	54408	CDS	29	ssDNA binding protein
54587	56899	Gene	30	
56944	59584	Gene	31	
59579	59584	Poly(A) signal		
56944	59550	CDS	31	gB, fusogen
59703	60150	Gene	32	
60145	60150	Poly(A) signal		
59703	60134	CDS	32	Substrate for ORF 47 kinase
62074	60245	Gene	33	
60250	60245	Poly(A) signal		
62074	60257	CDS	33	Protease
63846	62107	Gene	34	
64689	63913	CDS	35	
64300	64306	promoter		TATA element
64321	64325	5′end of dPyKmRNA		
64743	65800	Gene	36	
65795	65800	Poly(A) signal		
64743	65768	CDS	36	Thymidine kinase
65817	65821	3′end of dPyKmRNA		
66010	68552	Gene	37	
68747	68552	Poly(A) signal		
66010	68535	CDS	37	gH
70229	68583	Gene	38	
68588	68583	Poly(A) signal		
70229	68604	CDS	38	
70569	71305	Gene	39	
71300	71305	Poly(A) signal		
70569	71291	CDS	39	
71476	75699	Gene	40	
75694	75699	Poly(A) signal		
71476	75666	CDS	40	Major nucleocapsid protein
75783	76748	Gene	41	
76743	76748	Poly(A) signal		
75783	76733	CDS	41	
77974	76791	Gene	42	
76786	76791	Poly(A) signal		ORF 45+ORF 42
77974	76787	CDS	42	
78105	80136	Gene	43	
80131	80136	Poly(A) signal		
78105	80135	CDS	43	
80295	81449	Gene	44	
81444	81449	Poly(A) signal		
80295	81386	CDS	44	
82529	81474	CDS	45	
82654	83253	CDS	46	
83103	84635	CDS	47	Protein kinase, tegument protein
84602	86257	CDS	48	
86161	86429	Gene	49	
86424	86429	Poly(A) signal		
86161	86406	CDS	49	
87807	86466	Gene	50	
86471	86466	Poly(A) signal		
87807	86500	CDS	50	
87806	90313	CDS	51	Origin binding protein
90418	92771	Gene	52	
92766	92771	Poly(A) signal		
90418	92733	CDS	52	
93775	92775	Gene	53	
92780	92775	Poly(A) signal		
93775	92780	CDS	53	
95909	93600	CDS	54	
95921	98566	CDS	55	
98493	99280	Gene	56	
99275	99280	Poly(A) signal		
98493	99224	CDS	56	
99548	99309	Gene	57	
99314	99309	Poly(A) signal		
99548	99333	CDS	57	Cytoplasmic protein
100194	99529	CDS	58	
101141	100224	CDS	59	Uracil-DNA glycosylase
101574	101092	CDS	60	gL, chaperone for gH
104410	102926	Gene	61	
102931	102926	Poly(A) signal		
104410	103007	CDS	61	Transactivator, transrepressor
104849	104850	Miscellaneous		UL/IRL boundary
104938	104939	Miscellaneous		IRL/IRS boundary
109061	105065	Gene	62	
105071	105065	Poly(A) signal		
109061	105129	CDS	62	Transactivator, tegument protein
109693	109718	Repeat region		Reiteration 4
110017	110278	Origin of replication		Origin of replication
110507	111359	Gene	63	
111352	111357	Poly(A) signal		
110507	111343	CDS	63	Tegument protein
111491	112072	Gene	64	
112067	112072	Poly(A) signal		
111491	112033	CDS	64	
112571	112107	Gene	65	
112112	112107	Poly(A) signal		
112571	112263	CDS	65	Virion protein
112263	112264	Miscellaneous		IRS/US boundary
112968	114172	Gene	66	
114167	114172	Poly(A) signal		
112968	114149	CDS	66	Protein kinase
114427	115523	Gene	67	
115518	115523	Poly(A) signal		
114427	115491	CDS	67	gI
115739	117652	Gene	68	
117647	117652	Poly(A) signal		
115739	117610	CDS	68	gE
117498	117499	Miscellaneous		US/TRS boundary
118266	117690	Gene	69	
117495	117490	Poly(A) signal		
118266	117724	CDS	69	
119250	118400	Gene	70	
118405	118400	Poly(A) signal		
119250	118414	CDS	70	Tegument protein
119479	119742	Origin of replication		Origin of replication
119921	120066	Repeat region		Reiteration R4
120698	124694	Gene	71	
124689	124694	Poly(A) signal		
120698	124630	CDS	71	Transactivator, tegument protein

[a] CDS, coding sequence; dPyKmRNA, deoxypyrimidine kinase mRNA.
[b] ORF was annotated according to the work of Gomi et al. (20)

TABLE 2. Complete Oka-V_{Merck} genome annotation

Start	Stop	Feature[a]	ORF	Function or comment
88	89	Miscellaneous		TRL/UL boundary
914	587	Gene	1	
592	587	Poly(A) signal		
914	588	CDS	1	
1133	1861	Gene	2	
1133	1849	CDS	2	
1856	1861	Poly(A) signal		
2446	1889	Gene	3	
1894	1889	Poly(A) signal		
2446	1907	CDS	3	
4140	2781	Gene	4	
2781	2776	Poly(A) signal		
4140	2782	CDS	4	Transactivator, tegument protein
5273	4251	Gene	5	
5273	4251	CDS	5	gK
8576	5325	Gene	6	
8606	9398	Gene	7	
9393	9398	Poly(A) signal		
8606	9385	CDS	7	
10666	9425	Gene	8	
9430	9425	Poly(A) signal		
10666	9476	CDS	8	Deoxyuridine triphosphatase
10641	10904	CDS	9A[b]	gN
11008	11963	Gene	9	
11958	11963	Poly(A) signal		
11008	11916	CDS	9	Syncytium formation, virion protein
12159	13420	Gene	10	
13415	13420	Poly(A) signal		
12159	13391	CDS	10	Transactivator, tegument protein
13589	16076	Gene	11	
13936	14196	Repeat region		Reiteration R1
16071	16076	Poly(A) signal		
13589	16003	CDS	11	
16168	18153	Gene	12	
18695	19350	Gene	13	
19345	19350	Poly(A) signal		
18395	19300	CDS	13	
21067	19296	Gene	14	
19301	19296	Poly(A) signal		
20526	20851	Repeat region		Reiteration R2
21067	19385	CDS	14	
22432	21198	Gene	15	
21203	21198	Poly(A) signal		
22432	21212	CDS	15	
23748	22522	Gene	16	
24103	25468	Gene	17	
25463	25468	Poly(A) signal		
24103	25467	CDS	17	
26444	25501	Gene	18	
25506	25501	Poly(A) signal		
26444	25524	CDS	18	Ribonucleotide reductase, small subunit
28796	26469	Gene	19	Ribonucleotide reductase, big subunit
30426	28956	Gene	20	
28961	28956	Poly(A) signal		
30426	28975	CDS	20	
30710	33856	Gene	21	
33851	33856	Poly(A) signal		
30710	33826	CDS	21	Nucleocapsid
34034	42341	Gene	22	
41405	41470	Repeat region		Reiteration R3
42336	42341	Poly(A) signal		
34034	42325	CDS	22	
43088	42376	Gene	23	
42381	42376	Poly(A) signal		
43088	42381	CDS	23	
43971	43161	Gene	24	
43166	43161	Poly(A) signal		
43971	43162	CDS	24	
44568	44081	Gene	25	
44086	44081	Poly(A) signal		
44568	44098	CDS	25	
44456	46123	Gene	26	
46077	47193	Gene	27	
47188	47193	Poly(A) signal		
46077	47078	CDS	27	
50586	46981	Gene	28	
46986	46981	Poly(A) signal		
50586	47002	CDS	28	DNA polymerase
50807	54458	Gene	29	
54453	54458	Poly(A) signal		
50807	54406	CDS	29	Single-stranded-DNA binding protein
54585	56897	Gene	30	
56942	59582	Gene	31	
59577	59582	Poly(A) signal		
56942	59548	CDS	31	gB, fusogen
59701	60148	Gene	32	
60143	60148	Poly(A) signal		
59701	60132	CDS	32	Substrate for ORF 47 kinase
62071	60242	Gene	33	
60247	60242	Poly(A) signal		
62071	60254	CDS	33	Protease
63843	62104	Gene	34	
64686	63910	CDS	35	
64297	64303	Promoter		TATA element
64321	64325	5′ end of dPyKmRNA		
64740	65797	Gene	36	
65793	65797	Poly(A) signal		
64741	65765	CDS	36	Thymidine kinase
65814	65818	3′end of dPyKmRNA		
66007	68549	Gene	37	
68744	68549	Poly(A) signal		
66007	68532	CDS	37	gH
70226	68580	Gene	38	
68585	68580	Poly(A) signal		
70226	68601	CDS	38	
70566	71302	Gene	39	
71297	71302	Poly(A) signal		
70566	71288	CDS	39	
71473	75696	Gene	40	
75691	75696	Poly(A) signal		
71473	75663	CDS	40	Major nucleocapsid protein
75780	76745	Gene	41	
76740	76745	Poly(A) signal		
75780	76730	CDS	41	
77971	76788	Gene	42	
76783	76788	Poly(A) signal		ORF 45+ORF 42
77971	76784	CDS	42	
78102	80133	Gene	43	
80128	80133	Poly(A) signal		
78102	80132	CDS	43	
80292	81446	Gene	44	
81441	81446	Poly(A) signal		
80292	81383	CDS	44	
82526	81471	CDS	45	
82651	83250	CDS	46	
83100	84632	CDS	47	Protein kinase, tegument protein
84599	86254	CDS	48	
86158	86426	Gene	49	
86421	86426	Poly(A) signal		
86158	86403	CDS	49	
87804	86463	Gene	50	
86468	86463	Poly(A) signal		
87804	86497	CDS	50	
87803	90310	CDS	51	Origin binding protein
90415	92768	Gene	52	
92763	92768	Poly(A) signal		
90415	92730	CDS	52	
93772	92772	Gene	53	
92777	92772	Poly(A) signal		
93772	92777	CDS	53	
95906	93597	CDS	54	
95918	98563	CDS	55	
98490	99277	Gene	56	
99272	99277	Poly(A) signal		
98490	99221	CDS	56	
99545	99306	Gene	57	
99311	99306	Poly(A) signal		
99545	99330	CDS	57	Cytoplasmic protein
100191	99526	CDS	58	
101138	100221	CDS	59	Uracil-DNA glycosylase
101571	101089	CDS	60	gL, chaperone for gH
104407	102923	Gene	61	
102928	102923	Poly(A) signal		
104407	103004	CDS	61	Transactivator, transrepressor
104846	104847	Miscellaneous		UL/IRL boundary
104935	104936	Miscellaneous		IRL/IRS boundary
109058	105062	Gene	62	
105068	105062	Poly(A) signal		
109058	105126	CDS	62	Transactivator, tegument protein
109690	109715	Repeat region		Reiteration 4
110014	110277	Origin of replication		Origin of replication
110506	111356	Gene	63	
111351	111356	Poly(A) signal		
110506	111342	CDS	63	Tegument protein
111490	112067	Gene	64	
112062	112067	Poly(A) signal		
111490	112032	CDS	64	
112566	112102	Gene	65	
112107	112102	Poly(A) signal		
112566	112258	CDS	65	Virion protein
112258	112259	Miscellaneous		IRS/US boundary
112963	114167	Gene	66	
114162	114167	Poly(A) signal		
112963	114144	CDS	66	Protein kinase
114422	115518	Gene	67	
115513	115518	Poly(A) signal		
114422	115486	CDS	67	gI
115734	117647	Gene	68	
117642	117647	Poly(A) signal		
115734	117605	CDS	68	gE
117490	117491	Miscellaneous		US/TRS boundary
118260	117682	Gene	69	
117687	117682	Poly(A) signal		
118260	117718	CDS	69	
119244	118394	Gene	70	
118399	118394	Poly(A) signal		
119244	118408	CDS	70	Tegument protein
119473	119736	Origin of replication		Origin of replication
119915	120060	Repeat region		Reiteration R4
120692	124688	Gene	71	
124683	124688	Poly(A) signal		
120692	124624	CDS	71	Transactivator, tegument protein

[a] CDS, coding sequence; dPyKmRNA, deoxypyrimidine kinase mRNA.
[b] ORF was annotated according to the work of Gomi et al. (20).

available in GenBank under the accession numbers DQ008354 and DQ008355, respectively.

RESULTS

Oka-V_{GSK} and Oka-V_{Merck} genome organization. The full-length consensus sequence of Oka-V_{GSK} and Oka-V_{Merck} vaccine strains was essentially determined by bidirectional sequencing of overlapping PCR-amplified fragments. Occasionally, when the amplified region contained SNPs that could not be conclusively resolved, the amplified fragments were subcloned and a consensus sequence was derived from multiple plasmid clones. The obtained sequences were assembled and the complete genomes of the vaccines were annotated using the VZV sequence of the Dumas strain published by Davison and Scott as a template (12). The full annotations for Oka-V_{GSK} and Oka-V_{Merck} are presented in Tables 1 and 2, respectively.

The complete genomes of Oka-V_{GSK} and Oka-V_{Merck} strains are comprised of 124,821 and 124,815 bp, respectively. Like the wild-type Dumas strain and the parental Japanese Oka-V strain, the Oka-V_{GSK} and Oka-V_{Merck} genomes consist of a unique long region flanked by terminal repeat long and internal repeat long inverted repeat regions, as well as a unique short region flanked by internal repeat short (IRS) and terminal repeat short (TRS) inverted repeat regions. An origin of replication was found in both the IRS and TRS regions. Four unique reiteration regions (R1 to R4) were found along the genome, with R4 duplicated in the IRS and TRS regions.

All the open reading frames (ORFs) described for the Dumas VZV strain (12) and the Oka vaccine parental strain (22) were found in the two Oka-derived vaccine strains (Tables 1 and 2). The 72 ORFs predicted to encode proteins were evenly distributed on both DNA strands. Three genes were located within the repeat sequences and were therefore duplicated within the VZV genome, so that ORFs 69 to 71 in the IRS region correspond to ORFs 62 to 64 in the TRS region.

Comparison of Oka strain genomes to the Dumas strain genome. The obtained sequences of Oka-V_{GSK} and Oka-V_{Merck} were aligned with the full-length VZV genomes of Oka-P, Oka-V, and Dumas strains. All sequence differences between the four Oka strains and the Dumas strain are given in Table 3. A total of 326 nucleotide positions displaying differences relative to the genome of Dumas strain (X04370 [12]) were identified. Among these, 228 were common to the four Oka strains, and the remaining 98 were specific to one, two, or three of the Oka strains. Several deletions or insertions were found, but most mutations were substitutions of one nucleotide, i.e., SNPs. Frequently, the original nucleotide was nonetheless preserved, resulting in a mixture of two nucleotides present at the same position (Table 3). Because, to our knowledge, the vaccine strains were never cloned, this is consistent with the existence of multiple viral species that evolved during the attenuation process. Multiple SNPs were found to still contain the original Oka-P-specific nucleotide. This supports the cooperative effect of the overall pattern of nucleotide substitutions in the expression of the attenuation phenotype and, to a lesser extent, the contribution of individual SNPs.

The 98 differences between the Oka-V_{GSK} (124,821 bp), Oka-V_{Merck} (124,815 bp), Oka-P (125,125 bp), and Oka-V (125,078 bp) genomes were found in 25 ORFs (ORFs 1, 2, 6, 9A, 10, 11, 14, 18, 21, 22, 31, 35, 39, 45, 47, 48, 50, 51, 52, 54, 55, 62, 64, and 71), the R1 and R3 repeat regions (in ORFs 11 and 22, respectively), and one origin of replication (Table 3).

The total number of differences between the four Oka strains was determined (Table 4). Of the 98 differences identified, 69 were found between Oka-P and Oka-V, 51 between Oka-V and Oka-V_{GSK}, and 68 between Oka-V and Oka-V_{Merck}. Consequently, Oka-V_{Merck} contains 17 more differences that discriminate it from Oka-V compared with Oka-V_{GSK}.

Although the highest convergence was found for Oka-V_{GSK} and Oka-V_{Merck}, they still had 36 nucleotide differences (Table 4). For 12 of these positions, Oka-V_{Merck} had nucleotides matching the Oka-P strain, whereas the Oka-V_{GSK} strain had only a single position (119683) where the sequence was Oka-P-like. Overall, for the positions in which Oka-V_{GSK} differed from Oka-V_{Merck}, the Oka-V_{GSK} sequence was closer to Oka-V, whereas the Oka-V_{Merck} sequence was closer to Oka-P.

Sixty-nine nucleotide changes between the Oka-V and the Oka-P strains were identified (Table 5). Among these 69 differences, 56 positions in Oka-P were identical to the reference Dumas strain, whereas only 11 positions in Oka-V were identical to the Dumas strain. Identical nucleotides for many of these positions were also present in Oka-V_{GSK} and Oka-V_{Merck}.

To better characterize the observed differences, the substitution spectra were analyzed (Fig. 2). The large majority of mutations were SNPs and only partial, with two different nucleotides at the same position. Compared to Oka-P, transitions (i.e., mutations resulting in substitution of a purine for a purine [A↔ G] or a pyrimidine for a pyrimidine [C↔ T]) were more frequently (64% to 69%) observed for the Oka-V_{GSK} and Oka-V_{Merck} strains than transversions (i.e., mutations resulting in substitution of a purine for a pyrimidine and vice versa; 13% to 17%). Transversions were more common than insertions or deletions (≤10%). The majority of the identified mutations were silent mutations, either because they were located in intergenic regions or because of the degenerated genetic code. A significant proportion of mutations in intragenic regions (~45%) caused single amino acid substitutions in both the Oka-V_{GSK} and Oka-V_{Merck} strains (Fig. 2). No stop or frameshift mutations were identified. All deletions and insertions either were located in intergenic regions or, when located within coding regions, were multiples of three bases.

Comparison of Oka-V_{GSK} and Oka-V_{Merck} genomes. Sequence differences observed between the Oka-V_{GSK} and Oka-V_{Merck} strains are described in Table 6. Only 36 differences were found throughout the complete genomes (i.e., ~125 kb), three of which were repeated in ORF 62 and its duplicate, ORF 71. These 33 nucleotide unique position changes resulted in 14 amino acid changes, 1 each in ORFs 6, 9A, 10, 31, 39, and 52 and 2 each in ORFs 14, 55, and 62/71 and the R3 repeat region.

Among these 36 position differences between Oka-V_{GSK} and Oka-V_{Merck}, Oka-V_{GSK} had 23 nucleotide sequences identical to Oka-V but only 3 identical to Oka-P. In contrast, Oka-V_{Merck} had 18 positions identical to Oka-P but only 6 identical to Oka-V (Table 6 and Fig. 3).

TABLE 3. Comparison of complete genomic sequences of Dumas and Oka strains of VZV[a]

Feature relative to WT (Dumas)	Position (WT)	Feature in:				Position in:	
		Oka-P	Oka-V	Oka-V_{GSK}	Oka-V_{Merck}	Oka-V_{GSK}	Oka-V_{Merck}
A→G	1	X	X	X	X	1	1
G→C	3	X	X	X	X	3	3
Deletion of C (from WT)	109	X	X	X	X	109	109
G→C	178	-	-	X	X	177	177
A→G	236	X	X	X	X	235	235
C→T	262	X	X	X	X	261	261
T→C	560	-	X	X	X	559	559
G→A (ORF 1), N, silent	685	X	X	X	X	684	684
T→T/C (ORF 1), Q, silent	703	-	X	C	C	702	702
T→T/C (ORF 1), P, silent	763	-	X	C	C	762	762
T→C (ORF 1), T→A	789	X	X	X	X	788	788
T→C (ORF 1), Q→R	790	X	X	X	X	789	789
T→C (ORF 1), Q→R	791	X	X	X	X	790	790
C→G (ORF 2), G, silent	1838	-	X	-	-	1837	1837
T→T/C	2515	-	X	C	-	2514	2514
A→G (ORF 4), T, silent	3764	X	X	X	X	3763	3763
C→T (ORF 5), K, silent	4258	X	X	X	X	4257	4257
A→G (ORF 6), S→P	5745	-	X	X	A/G	5744	5744
G→T (ORF 6), H→Q	6853	X	X	X	X	6852	6852
C→A (ORF 6), G→V	7091	X	X	X	X	7090	7090
C→T (ORF 6), P, silent	7753	X	X	X	X	7752	7752
T→C	9460	X	X	X	X	9459	9459
G→A (ORF 8), P→S	10079	X	X	X	X	10078	10078
T→C/T (ORF 9A), W→R	10900	-	X	X	-	10899	10899
T→G (ORF 9), S, silent	11890	X	X	X	X	11889	11889
A→G (ORF 9), T→A	11906	X	X	X	X	11905	11905
C→A (ORF 10), P→H	12188	X	X	X	X	12187	12187
T→C (ORF 10), F→S	12284	X	X	X	X	12283	12283
T→C (ORF 10), F→S	12285	X	X	X	X	12284	12284
C→C/T (ORF 10), A→V	12779	-	X	X	-	12778	12778
T→G (ORF 10), G, silent	13173	X	X	X	X	13172	13172
G→A	13407	X	X	X	X	13406	13406
Deletion (ORF 11, R1), ATTGACGACGAGG GAGAGGCGGAGGA GGGAGAGGCGGA GGAGGGAGAGGC GGAGGAGGGA GAG, IDDEGEAEEG EAEEGEAEEGE	14088	X	X	X	X	14086	14086
Deletion (ORF 11, R1), GCGGAGGAGGAC GCG, AEEDA	14199–213	X	X	X	X	14134–48	14134–48
Insertion (ORF 11, R1), CGCGATCGACGAC GAGGGAGAGGCG GAGGAGGA	14242	X	-	X	X	14164–96	14164–96
T→C	14390	X	X	X	X	14344	14344
C→T (ORF 12), V, silent	17404	X	X	X	X	17358	17358
C→T (ORF 12), L, silent	17834	X	X	X	X	17788	17788
C→T (ORF 12), T, silent	18082	X	X	X	X	18036	18036
G→A (ORF 13), K, silent	18467	X	X	X	X	18421	18421
T→T/C (ORF 14), stop	19431	-	X	-	-	19385	19385
A→G (ORF 14), I, silent	19719	X	X	X	X	19673	19673
T→A (ORF 14), Y→F	20656	X	X	X	X	20610	20610
T→C (ORF 14), T→A	20684	X	X	X	X	20638	20638
C→C/T (ORF 14), K, silent	20703	-	-	X	-	20657	20657
A→T (ORF 14), E→V	20711	X	X	-	-	20665	20665
C→A (ORF 14), C→A	20745	X	X	-	-	20699	20699
T→A (ORF 14), T→S	20753	X	X	X	X	20707	20707

Continued on following page

TABLE 3—*Continued*

Feature relative to WT (Dumas)	Position (WT)	Feature in:				Position in:	
		Oka-P	Oka-V	Oka-V_{GSK}	Oka-V_{Merck}	Oka-V_{GSK}	Oka-V_{Merck}
C→A (ORF 14), K→N	20787	X	X	C/A	-	20741	20741
C→A (ORF 14), K→N	20829	X	X	C/A	C/A	20783	20783
T→A/T (ORF 14), T→S	20837	-	-	X	X	20791	20791
C→A (ORF 14), K→N	20871	-	-	C/A	C/A	20825	20825
A→T (ORF 14), S→T	20879	-	-	A/T		20833	20833
C→A (ORF 14), K→N	20913	-	-	A/C	A/C	20867	20867
T→A (ORF 14), T→S	21005	X	X	X	X	20959	20959
G→A (ORF 15), L, silent	21371	X	X	X	X	21325	21325
G→T (ORF 15), R, silent	21734	X	X	X	X	21688	21688
G→A (ORF 15), S, silent	22311	X	X	X	X	22265	22265
A→G	22504	X	X	X	X	22458	22458
A→G (ORF 16), M→T	22794	X	X	X	X	22748	22748
A→G (ORF 16), F, silent	23294	X	X	X	X	23248	23248
Deletion (ORF 17), dCAT (delS)	24516	X	X	X	X	24469	24469
A→G (ORF 17), T→A	24578	X	X	X	X	24529	24529
C→T (ORF 17), T→M	24654	X	X	X	X	24605	24605
G→A (ORF 17), V→I	25067	X	X	X	X	25018	25018
A→G (ORF 18), N, silent	26125	-	X	X	A/G	26076	26076
A→G (ORF 19), H, silent	27523	X	X	X	X	27474	27474
T→G (ORF 20), G, silent	29201	X	X	X	X	29152	29152
C→T/C (ORF 21), T→I	31732	-	X	-	-	31683	31683
A→G (ORF 21), T→A	32274	X	X	X	X	32225	32225
T→C (ORF 21), H, silent	33722	X	X	X	X	33673	33673
T→C (ORF 21), D, silent	33725	X	X	X	X	33676	33676
T→C (ORF 21), N, silent	33728	X	X	X	X	33679	33679
T→C (ORF 22), V, silent	35543	X	X	X	X	35494	35494
A→G (ORF 22), L, silent	37649	X	X	X	X	37600	37600
A→G (ORF 22), I→V	37902	X	X	X	X	37853	37853
T→C (ORF 22), T, silent	38036	-	-	C/T	C/T	37987	37987
T→C (ORF 22), Y→H	38055	X	X	X	X	38006	38006
A→C (ORF 22), P, silent	38081	X	X	X	X	38032	38032
G→A (ORF 22), E, silent	38177	X	X	X	X	38128	38128
G→T (ORF 22), T, silent	38714	X	X	X	X	38665	38665
C→T (ORF 22), A, silent	38717	X	X	X	X	38668	38668
A→G (ORF 22), R, silent	39023	X	X	X	X	38974	38974
T→T/G (ORF 22), P, silent	39227	-	X	X	-	39178	39178
G→A (ORF 22), Q, silent	39263	X	X	X	X	39214	39214
G→A (ORF 22), R→H	39394	X	X	X	X	39345	39345
A→G (ORF 22), V, silent	39530	X	X	X	X	39481	39481
A→G (ORF 22), Q, silent	40388	X	X	X	X	40339	40339
T→C (ORF 22), P, silent	41057	X	X	X	X	41008	41008
G→A	41452	X	X	X	X	41403	41403
C→T (R3 repeat), A→V	41458	X	X	X	X	41409	41409
G→C (R3 repeat), A→V	41459	X	X	X	X	41410	41410
C→T (R3 repeat), A→V	41476	X	-	X	X	41427	41427
Deletion, GCGCAGCCC	41475–83	-	X	-	-	41426–34	41426–34
G→C (R3 repeat), A→V	41476	X	-	X	X	41427	41427
Deletion, GCGCAGCCC GCGCAGACCGTCC AGCCCGCGCAG CCC, AQPAQTVQ PAQP	41484–519	X	X	-	-	41435–70	41435–70

Continued on following page

TABLE 3—*Continued*

Feature relative to WT (Dumas)	Position (WT)	Feature in:				Position in:	
		Oka-P	Oka-V	Oka-V_{GSK}	Oka-V_{Merck}	Oka-V_{GSK}	Oka-V_{Merck}
C→T (R3 repeat), A→V	41485	-	-	X	-	41436	41436
C→C/T (R3 repeat), A→V	41494	-	-	X	-	41445	41445
A→C (R3 repeat), T→P	41499	-	-	X	X	41450	41450
C→T (ORF 22), T, silent	41618	X	X	X	X	41569	41569
G→A (ORF 22), S→N	41764	X	X	X	X	41715	41715
C→G (ORF 22), Q→E	42069	X	X	X	X	42020	42020
C→T (ORF 22), R, silent	42176	X	X	X	X	42127	42127
A→C (ORF 22), A, silent	42242	X	X	X	X	42193	42193
	42403	Del AAA	Del AA	Ins A	Del A	42355	42353
T→G (ORF 23), S, silent	42476	X	X	X	X	42428	42426
T→C (ORF 24), I→V	43262	X	X	X	X	43214	43212
C→T (ORF 26), C, silent	44835	X	X	X	X	44787	44785
A→G (ORF 28), C→R	47162	X	X	X	X	47114	47112
C→T (ORF 28), L, silent	47940	X	X	X	X	47892	47890
T→C (ORF 28), S→G	48050	X	X	X	X	48002	48000
G→A (ORF 28), T, silent	48825	X	X	X	X	48777	48775
G→A (ORF 28), L, silent	49535	X	X	X	X	49487	49485
C→A (ORF 28), G→C	50081	X	X	X	X	50033	50031
C→T (ORF 29), S, silent	51168	X	X	X	X	51120	51118
A→G (ORF 29), Q, silent	52917	X	X	X	X	52869	52867
A→C (ORF 29), I→L	53482	X	X	X	X	53434	53432
G→A (ORF 29), A→T	53938	X	X	X	X	53890	53888
Deletion (ORF 29), ACA TTTCAGGGTCAA, NISGS	54359–73	X	X	X	X	54310	54308
Deletion, T	54562	X	X	X	X	54498	54496
T→C	54564	X	X	X	X	54500	54498
A→G (ORF 30), P, silent	55820	X	X	X	X	55756	55754
A→C (ORF 31), T→P	57224	X	X	X	X	57160	57158
A→C (ORF 31), A, silent	57301	X	X	X	X	57237	57235
G→T (ORF 31), A, silent	57397	X	X	X	X	57333	57331
A→G (ORF 31), I→V	58595	-	A/G	A/G	X	58531	58529
A→A/G (ORF 31), P, silent	59287	-	X	X	X	59223	59221
Insertion, G	59760	X	X	X	X	59697	59695
Deletion	60278	Del5A	Del5A	Del A	Del AA	60214	60211
A→C	60279	X	X	X	X	60215	60212
C→A (ORF 33), A, silent	60405	X	X	X	X	60341	60338
T→G (ORF 33), Y→S	60781	X	X	X	X	60717	60714
G→A (ORF 33), P→L	61018	X	X	X	X	60954	60951
G→A (ORF 33), P→L	61019	X	X	X	X	60955	60952
T→C (ORF 33), N→G	61201	X	X	X	X	61137	61134
T→C (ORF 33), N→G	61202	X	X	X	X	61138	61135
A→G (ORF 35), A, silent	64067	-	X	X	A/G	64003	64000
A→G (ORF 35), C, silent	64136	X	X	X	X	64072	64069
T→C (ORF 35), P, silent	64259	X	X	X	X	64195	64192
T→C (ORF 35), M→V	64375	X	X	X	X	64311	64308
C→T (ORF 36), A, silent	64989	X	X	X	X	64925	64922
C→T (ORF 36), S→L	65669	X	X	X	X	65605	65602
G→T (ORF 37), L, silent	66646	X	X	X	X	66582	66579
C→T (ORF 37), P→L	66879	X	X	X	X	66815	66812
G→A (ORF 37), R→K	68172	X	X	X	X	68108	68105
A→G (ORF 38), T, silent	69349	X	X	X	X	69285	69282
T→C (ORF 38), S→G	69756	X	X	X	X	69692	69689

Continued on following page

TABLE 3—*Continued*

Feature relative to WT (Dumas)	Position (WT)	Feature in:				Position in:	
		Oka-P	Oka-V	Oka-V_{GSK}	Oka-V_{Merck}	Oka-V_{GSK}	Oka-V_{Merck}
T→C (ORF 39), M→T	71252	-	X	X	C/T	71188	71185
C→T (ORF 40), V, silent	72997	X	X	X	X	72933	72930
T→C (ORF 40), T, silent	73993	X	X	X	X	73929	73926
C→T (ORF 41), V, silent	76530	X	X	X	X	76466	76463
Deletion, T	78144	X	X	X	X	78079	78076
G→T	80244	X	X	X	X	80179	80176
A→G (ORF 44), N→D	80840	X	X	X	X	80775	80772
C→T (ORF 44), A, silent	81187	X	X	X	X	81122	81119
A→A/G (ORF 45), P, silent	82225	-	X	-	-	82160	82157
G→A/G (ORF 47), E, silent	84091	-	X	-	-	84026	84023
A→G (ORF 47), T, silent	84616	X	X	X	X	84551	84548
G→A (ORF 48), R→H	84983	X	X	X	X	84918	84915
C→T (ORF 48), D, silent	85563	X	X	X	X	85498	85495
A→A/G (ORF 48), T→A	85594	-	-	X	X	85529	85526
C→A (ORF 48), Q→K	86170	X	X	X	X	86105	86102
Deletion, CCTGAT AAAC	86484–93	X	X	X	X	86418	86415
T→G	86556	X	X	X	X	86481	86478
A→A/G (ORF 50), C, silent	87280	-	X	-	-	87205	87202
T→C/T (ORF 50), S→G	87306	-	X	-	-	87231	87228
C→T (ORF 50), S, silent	87841	X	X	X	X	87766	87763
G→T (ORF 51), S, silent	88477	X	X	X	X	88402	88399
A→G (ORF 51), T, silent	89734	-	X	X	-	89659	89656
T→C (ORF 51), T, silent	89905	X	X	X	X	89830	89827
G→T (ORF 51), Q→H	90202	X	X	X	X	90127	90124
T→C (ORF 51), S, silent	90217	X	X	X	X	90142	90139
G→A	90392	X	X	X	X	90317	90314
A→A/G (ORF 52), I→V	90535	-	X	X	-	90460	90457
C→T (ORF 52), G, silent	91191	X	X	X	X	91116	91113
A→G (ORF 52), T→A	92026	X	X	X	X	91951	91948
A→G (ORF 52), T→A	92092	X	X	X	X	92017	92014
A→G (ORF 52), H→R	92375	X	X	X	X	92300	92297
T→C (ORF 53), V, silent	92999	X	X	X	X	92924	92921
T→C (ORF 54), L, silent	94167	-	X	X	T/C	94092	94089
A→G (ORF 54), V, silent	94632	X	X	X	X	94557	94554
A→T (ORF 54), T, silent	94641	X	X	X	X	94566	94563
T→C (ORF 54), G, silent	95241	X	X	X	X	95166	95163
G→A (ORF 54), L, silent	95546	X	X	X	X	95471	95468
T→G (ORF 54), E→D	95601	X	X	X	X	95526	95523
T→C (ORF 55), L, silent	97141	X	X	X	X	97066	97063
T→T/C (ORF 55), V→A	97479	-	-	-	X	97404	97401
C→T (ORF 55), I, silent	97591	X	X	X	X	97516	97513
G→A/G (ORF 55), A→T	97748	-	X	X	X	97673	97670
T→C/T (ORF 55), C→R	97796	-	X	X	-	97721	97718
T→C (ORF 55), G, silent	98437	X	X	X	X	98362	98359
T→C (ORF 56), V, silent	98765	X	X	X	X	98690	98687
A→C (ORF 56), T, silent	98807	X	X	X	X	98732	98729
Deletion (ORF 56), TTC, S	99227–29	X	X	X	X	99148	99145
T→G (ORF 57), H→P	99421	X	X	X	X	99343	99340
A→G (ORF 58), Y, silent	99709	X	X	X	X	99631	99628
C→T (ORF 58), V→I	99981	X	X	X	X	99903	99900
T→A (ORF 58), K→N	100114	X	X	X	X	100036	100033
T→G (ORF 58), N→T	100151	X	X	X	X	100073	100070
A→G	100283	X	X	X	X	100205	100202
A→A/G (ORF 59), L→P	101089	X	X	X	X	101011	101008
C→T (ORF 60), A→T	101331	X	X	X	X	101253	101250

Continued on following page

TABLE 3—*Continued*

Feature relative to WT (Dumas)	Position (WT)	Feature in:				Position in:	
		Oka-P	Oka-V	Oka-V_{GSK}	Oka-V_{Merck}	Oka-V_{GSK}	Oka-V_{Merck}
Insertion (ORF 60), ATC	101623	X	X	X	X	101543–101545	101540–101542
T→C	101886	X	X	X	X	101811	101808
C→T	101991	X	X	X	X	101916	101913
G→A	102192	X	X	X	X	102117	102114
A→G	102203	X	X	X	X	102128	102125
Insertion, TCAAGCTTT AAAAACGTACCCCA AACTTAAAACGCTC AAATTGCCTTTTGG AGGCCTGCCCAACG GCCATTATCCCTTG GATCTAAGATTGAT TTGCGGTAACGTTT GCCAA	102219	X	X	-	-	102144	102141
C→A	102309	X	X	X	X	102234	102231
A→C	102351	X	X	X	X	102276	102273
A→G	102458	X	X	X	X	102383	102380
T→G	102601	X	X	X	X	102526	102523
T→C	103043	X	X	X	X	102968	102965
A→G	104898	X	X	X	X	104823	104820
C→G	105010	X	X	X	X	104935	104932
T→C	105012	X	X	X	X	104937	104934
T→C	105015	X	X	X	X	104940	104937
T→C	105017	X	X	X	X	104942	104939
Insertion, C	105020	X	-	X	X	104946	104943
Deletion, G	105054	X	X	X	X	104979	104976
Deletion, G	105071	X	X	X	X	104995	104992
Insertion, ACAA	105145	X	X	X	X	105075	105072
A→A/G	105169	-	X	X	X	105097	105094
A→A/G (ORF 62), L→S	105310	-	X	X	X	105238	105235
A→G (ORF 62), G, silent	105312	X	X	X	X	105240	105237
T→C (ORF 62), I→V	105356	-	X	X	T/C	105284	105281
A→G (ORF 62), L→P	105451	X	X	X	X	105379	105376
A→C (ORF 62), S→A	105512	X	X	X	X	105440	105437
A→G (ORF 62), V→A	105544	-	X	X	X	105472	105469
T→C (ORF 62), A, silent	105705	-	X	X	X	105633	105630
T→C (ORF 62), R→G	106262	-	X	X	X	106190	106187
T→C (ORF 62), A, silent	107136	-	X	X	T/C	107064	107061
C→T (ORF 62), A→T	107165	X	X	X	X	107093	107090
T→C (ORF 62), S→G	107252	-	X	X	X	107180	107177
T→C (ORF 62), R, silent	107307	X	X	X	X	107235	107232
A→A/G (ORF 62), V→A	107599	-	X	X	-	107527	107524
C→A (ORF 62), T, silent	107607	X	X	X	X	107535	107532
T→C (ORF 62), A, silent	107715	X	X	X	X	107643	107640
T→C (ORF 62), P, silent	108111	-	X	X	X	108039	108036
A→G (ORF 62), L, silent	108747	X	X	X	X	108675	108672
A→A/G (ORF 62), M→T	108838	-	X	X	X	108766	108763
G→A (ORF 62), H, silent	108951	X	X	X	X	108879	108876
C→G (ORF 62), A, silent	109044	X	X	X	X	108972	108969
A→A/G	109137	-	X	X	X	109065	109062
A→A/G	109200	-	X	X	-	109128	109125
T→C/T	109546	-	-	X	-	109474	109471
G→T	109654	X	X	X	X	109582	109579
Insertion, CAT	109696	X	X	X	X	109625–109627	109622–109624
Insertion, GGGAGGGG GCGCGGTACCCCGC CGATGGGGAGGGG GCGCGGTACCCCGC CGATGGGGAGGGG GCGCGGTACCCCGC CGATGGGGAGGGG GCGCGGTACCCCGC CGATG	109907	X	X	-	-	109838	109835
Insertion, GGGAGGGG GCGCGGTACCCCGC CGATG	109907	X	-	-	-	109838	109835

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TABLE 3—*Continued*

Feature relative to WT (Dumas)	Position (WT)	Feature in:				Position in:	
		Oka-P	Oka-V	Oka-V_{GSK}	Oka-V_{Merck}	Oka-V_{GSK}	Oka-V_{Merck}
G→A	110003	X	X	X	X	109934	109931
Deletion, G	110058	X	X	X	X	109988	109985
G→A (Ori), -, silent	110112	X	X	X	X	109934	109931
Deletion, AT	110212	-	X	X	-	110142	110140–110141
T→G	110214	-	-	-	X		110141
Insertion, ATATAG	110214	X	-	-	-	110142	110141
T→G (Ori)	110216	X	X	X	X	110144	110143
T→G (Ori)	110218	X	X	X	X	110146	110145
T→G (Ori)	110220	X	X	X	X	110148	110147
T→G (Ori)	110222	X	X	X	X	110150	110149
T→G (Ori)	110224	X	X	X	X	110152	110151
T→G (Ori)	110226	X	X	X	X	110154	110153
A→G (Ori)	110232	X	X	X	X	110160	110159
A→G (Ori)	110235	X	X	X	X	110163	110162
Deletion, GC	110378–110379	X	X	X	X	110305	110304
A→G (ORF 63), T, silent	111312	X	X	X	X	110238	110237
A→G (ORF 64), Q→R	111650	-	X	A/G	A/G	111576	111575
T→C (ORF 64), Y→H	112093	X	X	X	X	112019	112018
Deletion/insertion	112128	Del A	Del A	Ins 5a	Ins A	112064–112068	112063
G→A	112198	X	X	X	X	112129	112124
A→G (ORF 66), S, silent	114140	X	X	X	X	114071	114066
G→A (ORF 67), P, silent	115041	X	X	X	X	114072	114967
C→T (ORF 68), T→I	115926	X	X	X	X	115857	115852
C→T	117699	X	X	X	X	117630	117625
Deletion/insertion	117769	Del T	Del T	Ins 5T	Ins T	117701–117705	117696
A→G (ORF 69), Y→H	117804	X	X	X	X	117740	117731
T→C (ORF 69), Q→R	118247	-	X	T/C	T/C	118183	118174
T→C (ORF 70), T, silent	118585	X	X	X	X	118521	118512
Deletion, GC	119518–119519	X	X	X	X	119453	119444
Insertion, CTCTCT	119654	X	X	-	-	119588	119579
T→C (Ori)	119656	X	X	-	-	119590	119581
T→C (Ori)	119665	X	X	X	X	119599	119590
A→C (Ori)	119671	X	X	X	X	119605	119596
A→C (Ori)	119673	X	X	X	X	119607	119598
A→C (Ori)	119675	X	X	X	X	119609	119600
A→C (Ori)	119677	X	-	X	X	119611	119602
Deletion, ATATATAT	119677–119684	-	X	-		119611–119618	119602–119609
A→C (Ori)	119679	X	-	X	X	119613	119604
A→C (Ori)	119681	X	-	X	X	119615	119606
A→C (Ori)	119683	X	-	X	A/C	119617	119608
C→T (Ori)	119785	X	X	X	X	119719	119710
Deletion, C	119847	X	X	X	X	119780	119771
C→T,	119894	X	X	X	X	119827	119818
Insertion, TACCGCGCC CCCTCCCCATCGGC GGGGTACCGCGCCC CCTCCCCATCGGCG GGGTACCGCGCCCC CTCCCCATCGGCGG GGTACCGCGCCCCC TCCCCATCGGCGGG GTACCGCGCCCCCT CCCCATCGGCGGGG	120135	X	X	-	-	120068	120059
Insertion, TACCGCGCC CCCTCCCCATCGGC GGGG	120135	X	-	-		120068	120060
Insertion, GAT	120202	X	X	X	X	120136–120138	120127–120129
C→A	120243	X	X	X	X	120179	120170
A→A/G	120351	-	-	X	-	120287	120278
T→T/C	120697	-	X	X	-	120633	120624
T→T/C	120760	-	X	X	X	120696	120687
G→C (ORF 71), A, silent	120853	X	X	X	X	120789	120780
C→T (ORF 71), H, silent	120946	X	X	X	X	120882	120873
T→C/T (ORF 71), M→T	121059	-	X	X	X	120995	120986
T→C (ORF 71), L, silent	121150	X	X	X	X	121086	121077
A→G (ORF 71), P, silent	121786	-	X	X	X	121722	121713

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TABLE 3—*Continued*

Feature relative to WT (Dumas)	Position (WT)	Feature in:				Position in:	
		Oka-P	Oka-V	Oka-V_{GSK}	Oka-V_{Merck}	Oka-V_{GSK}	Oka-V_{Merck}
A→G (ORF 71), A, silent	122182	X	X	X	X	122118	122109
G→T (ORF 71), T, silent	122290	X	X	X	X	122226	122217
T→C/T (ORF 71), V→A	122298	-	X	X	-	122234	122225
A→G (ORF 71), R, silent	122590	X	X	X	X	122526	122517
A→G (ORF 71), S→G	122645	-	X	X	X	122581	122572
G→A (ORF 71), A→T	122732	X	X	X	X	122668	122659
A→G (ORF 71), A, silent	122761	-	X	X	A/G	122697	122688
A→G (ORF 71), R→G	123635	-	X	X	X	123571	123563
A→G (ORF 71), A, silent	124192	-	X	X	X	124128	124119
T→C (ORF 71), V→A	124353	-	X	X	X	124289	124280
T→G (ORF 71), S→A	124385	X	X	X	X	124321	124312
T→C (ORF 71), L→P	124446	X	X	X	X	124382	124373
A→G (ORF 71), I→V	124541	-	X	X	A/G	124477	124468
T→C (ORF 71), G, silent	124585	X	X	X	X	124521	124512
T→C (ORF 71), L→S	124587	-	T/C	T/C	T/C	124523	124514
T→T/C	124728	-	X	X	X	124664	124655
Insertion, TGTT	124750	X	X	X	X	124687–124690	124678–124681
Deletion, C	124834	X	X	X	X	124773	124764
Deletion, C	124851	X	X	X	X	124789	124780
A→G	124880	NA	NA	X	X	124818	124809
A→G	124882	NA	NA	X	X	124820	124811

[a] A partial analysis of 20 of these nucleotide differences was published previously (70). Nucleotide positions within ORFs are indicated, as well as the encoded amino acids. Ori, origin of replication; WT, wild type. X, difference relative to Dumas strain; -, identical nucleotide relative to Dumas strain; NA, not applicable; Del, deletion; Ins, insertion. Where applicable, the resulting codon switch is specified.

DISCUSSION

In this study, we compared the complete genomes of the varicella vaccine strains Oka-V_{GSK} and Oka-V_{Merck}, both derived from the original attenuated Oka-V strain (67). Phylogenetic analyses of these sequences along with 16 other complete VZV genomes were recently reported (50, 69), providing new insight into strain variability (69) and evidence of recombination between major circulating VZV clades (50).

Although VZV is a monotypic virus with a very low rate of interstrain sequence variations (0.061%) compared to other members of the *Herpesviridae* family of viruses (between 0.32% and 3.0% [47]), the sequence analysis of the Oka vaccine strains is not straightforward due to the presence of heterogeneous genomes with distinct sequences (21). Therefore, consensus sequencing provides only an indication of the most prevalent bases for each position. In the present study, we determined the full-length sequences of both Oka-V_{GSK} and Oka-V_{Merck} largely by bidirectional sequencing of overlapping PCR fragments, but when direct sequencing did not generate results of sufficient quality, fragments were subcloned and the consensus sequence was derived from numerous plasmid clones. All sequences obtained were confirmed on both DNA strands. This approach gave a high-quality assessment of the whole genomes of Oka-V_{GSK} and Oka-V_{Merck}, and this is, to our knowledge, the first published comparative analysis of the complete genomes of these two strains.

TABLE 4. Numbers of genomic sequence differences between the four Oka strains

Strain	No. of differences from:		
	Oka-V	Oka-V_{GSK}	Oka-V_{Merck}
Oka-P	69 (Table 5)	79	64
Oka-V		51	68
Oka-V_{GSK}			36 (Table 6)

Comparison with partial sequencing information published on these strains and the other Oka strains, Oka-P and Oka-V, is shown in Table 7 (3, 22, 32, 59, 60, 63, 69). Argaw et al. sequenced approximately 34 kb from the 3′ ends of Oka-V, Oka-P, and Oka-V_{Merck} strains, and Schmidt et al. sequenced approximately 26 kb of the Oka-V_{GSK} strain (3, 60). Two sequence differences were found for Oka-V_{Merck} in ORF 59 (position 101089; A versus A/G) and ORF 62 (position 105310; G versus A/G) (3). Six differences were observed between the present results and those previously published for Oka-V_{GSK}, and 13 differences were observed for Oka-V_{Merck} (60). Finally, comparison between the present study and a previous one (32) revealed quantitative (number of sequence differences between Oka-V_{GSK} and Oka-V_{Merck} strains) and qualitative (ORFs involved) discrepancies.

Our analysis of Oka-V_{GSK} and Oka-V_{Merck} sequences revealed that they have very few nucleotide differences. When we compared their complete genomic sequences (i.e., ~125 kb), we found that only 36 positions were different between Oka-V_{GSK} and Oka-V_{Merck}. These differences lead to 14 unique amino acid substitutions, which suggests that although these two vaccine strains are not identical, they are very similar. The differences resulting in amino acid substitutions were found in 10 different ORFs and in the R3 repeat region, while silent

TABLE 5. Comparison of complete genomic sequences of Oka-P and Oka-V strains of VZV[a]

Feature relative to WT (Dumas)	Position (WT)	Feature in:				Position in:	
		Oka-P	Oka-V	Oka-V_{GSK}	Oka-V_{Merck}	Oka-V_{GSK}	Oka-V_{Merck}
T→C	560	-	**X**	**X**	**X**	559	559
T→T/C (ORF 1), Q, silent	703	-	X	C	C	702	702
T→T/C (ORF 1), P, silent	763	-	X	C	C	762	762
C→G (ORF 2), G, silent	1838	-	X	-	-	1837	1837
T→T/C	2515	-	X	C	-	2514	2514
A→G (ORF 6), S→P	5745	-	**X**	**X**	A/G	5744	5744
T→C/T (ORF 9A), W→R	10900	-	**X**	**X**	-	10899	10899
C→C/T (ORF 10), A→V	12779	-	**X**	**X**	-	12778	12778
Insertion (ORF 11, R1), CGCGATCGA CGACGAGGGAGAGGCGGAGG AGGA	14242	X		X	X	14164–14196	14164–14196
T→T/C (ORF 14), stop	19431	-	X	-	-	19385	19385
A→G (ORF 18), N, silent	26125	-	**X**	**X**	A/G	26076	26076
C→T/C (ORF 21), T→I	31732	-	X	-	-	31683	31683
T→T/G (ORF 22), P, silent	39227	-	**X**	**X**	-	39178	39178
C→T (R3 repeat), A→V	41476	X		X	X	41427	41427
Deletion, GCGCAGCCC	41475–83	-	X	-	-	41426–41434	41426–41434
G→C (R3 repeat), A→V	41476	X		X	X	41427	41427
Deletion/insertion	42403	Del AAA	Del AA	Ins A	Del A	42355	42353
A→G (ORF 31), I→V	58595	-	**A/G**	**A/G**	X	58531	58529
A→A/G (ORF 31), P, silent	59287	-	**X**	**X**	**X**	59223	59221
A→G (ORF 35), A, silent	64067	-	**X**	**X**	A/G	64003	64000
T→C (ORF 39), M→T	71252	-	**X**	**X**	C/T	71188	71185
A→A/G (ORF 45), P, silent	82225	-	X	-	-	82160	82157
G→A/G (ORF 47), E, silent	84091	-	X	-	-	84026	84023
A→A/G (ORF 50), C, silent	87280	-	X	-	-	87205	87202
T→C/T (ORF 50), S→G	87306	-	X	-	-	87231	87228
A→G (ORF 51), T, silent	89734	-	**X**	**X**	-	89659	89656
A→A/G (ORF 52), I→V	90535	-	**X**	**X**	-	90460	90457
T→C (ORF 54), L, silent	94167	-	**X**	**X**	T/C	94092	94089
G→A/G (ORF 55), A→T	97748	-	**X**	**X**	**X**	97673	97670
T→C/T (ORF 55), C→R	97796	-	**X**	**X**	-	97721	97718
Insertion, C	105020	X		X	X	104946	104943
A→A/G	105169	-	**X**	**X**	**X**	105097	105094
A→A/G (ORF 62), L→S	105310	-	**X**	**X**	**X**	105238	105235
T→C (ORF 62), I→V	105356	-	**X**	**X**	T/C	105284	105281
A→G (ORF 62), V→A	105544	-	**X**	**X**	**X**	105472	105469
T→C (ORF 62), A, silent	105705	-	**X**	**X**	**X**	105633	105630
T→C (ORF 62), R→G	106262	-	**X**	**X**	**X**	106190	106187
T→C (ORF 62), A, silent	107136	-	**X**	**X**	T/C	107064	107061
T→C (ORF 62), S→G	107252	-	**X**	**X**	**X**	107180	107177
A→A/G (ORF 62), V→A	107599	-	**X**	**X**	-	107527	107524
T→C (ORF 62), P, silent	108111	-	**X**	**X**	**X**	108039	108036
A→A/G (ORF 62), M→T	108838	-	**X**	**X**	**X**	108766	108763
A→A/G	109137	-	**X**	**X**	**X**	109065	109062
A→A/G	109200	-	**X**	**X**		109128	109125
Insertion, GGGAGGGGGCGCGGTAC CCCGCCGATG	109907	X	-	-	-	109838	109835
Deletion, AT	110212	-	**X**	**X**	-	110142	110140–110141
Insertion, ATATAG	110214	X	-	-	-	110142	110141
A→G (ORF 64), Q→R	111650	-	**X**	A/G	A/G	111576	111575
T→C (ORF 69), Q→R	118247	-	**X**	T/C	T/C	118183	118174
A→C (Ori)	119677	X	-	X	X	119611	119602
Deletion, ATATATAT	119677–119684	-	X	-	-	119611–119618	119602–119609
A→C (Ori)	119679	X	-	X	X	119613	119604
A→C (Ori)	119681	X	-	X	X	119615	119606
A→C (Ori)	119683	X	-	X	A/C	119617	119608
Insertion, TACCGCGCCCCCTCCCCA TCGGCGGGG	120135	X	-	-	-	120068	120060
T→T/C	120697	-	**X**	**X**		120633	120624
T→T/C	120760	-	**X**	**X**	**X**	120696	120687
T→C/T (ORF 71), M→T	121059	-	**X**	**X**	**X**	120995	120986
A→G (ORF 71), P, silent	121786	-	**X**	**X**	**X**	121722	121713
T→C/T (ORF 71), V→A	122298	-	**X**	**X**		122234	122225
A→G (ORF 71), S→G	122645	-	**X**	**X**	**X**	122581	122572
A→G (ORF 71), A, silent	122761	-	**X**	**X**	A/G	122697	122688
A→G (ORF 71), R→G	123635	-	**X**	**X**	**X**	123571	123563
A→G (ORF 71), A, silent	124192	-	**X**	**X**	**X**	124128	124119
T→C (ORF 71), V→A	124353	-	**X**	**X**	**X**	124289	124280
A→G (ORF 71), I→V	124541	-	**X**	**X**	A/G	124477	124468
T→C (ORF 71), L→S	124587	-	**T/C**	**T/C**	**T/C**	124523	124514
T→T/C	124728	-	**X**	**X**	**X**	124664	124655

[a] Ori, origin of replication; WT, wild type. X, difference relative to Dumas strain; -, identical nucleotide relative to Dumas strain; Del, deletion; Ins, insertion. Where applicable, the resulting codon switch is specified. Boldface highlights homologies between genomic sequences of Oka-V and genomic sequences of Oka-V_{GSK} and/or Oka-V_{Merck}.

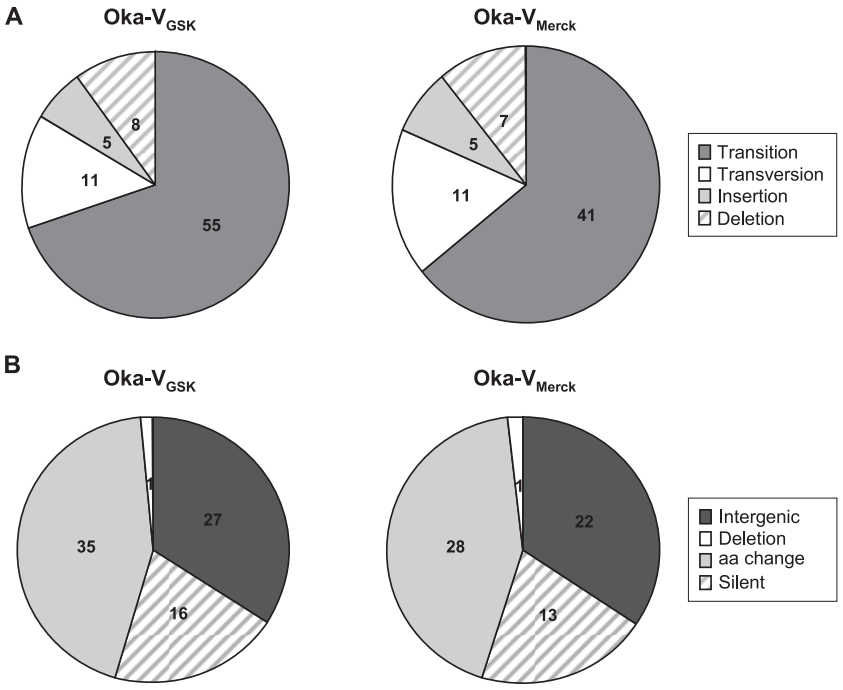

FIG. 2. Type (A) and function (B) of the mutations between Oka-P and the Oka-V_{GSK} and Oka-V_{Merck} vaccine strains of VZV. The numbers indicate the number of events identified for each category of mutations. aa, amino acid.

nucleotide substitutions were found in 8 different ORFs, and 1 noncoding substitution was found in the origin of replication.

Transactivation. ORF 62 encodes immediate early protein 62 (IE62), also known as a transcription regulator, which is the major component of the virion tegument and an important transactivating protein for all classes of VZV promoters (28, 49, 56). It is located in the short repeat sequences and has therefore a duplicate gene, ORF 71. These two duplicated genes cover 7% of the whole VZV genome. Recent studies suggested that ORF 62 could play a central role in the attenuated phenotype of the Oka vaccine strains (3, 20–22). Defined amino acid substitutions in ORF 62 that are associated with individual virus variants purified from the vaccine mixture have been linked to enhanced virus growth and spread in monolayer cell culture (22).

The present analysis of vaccine strains confirmed that a high number of mutations could be detected within ORF 62 (20–22). As previously discussed, these SNPs in ORF 62/71 may be important for attenuation of VZV (50, 69). The current analysis identified a nucleotide transition (position 105356 in ORF 62, corresponding to 124541 in ORF 71) that altered an Ile of the IE62 protein to a Val only partially in Oka-V_{Merck} and completely in Oka-V_{GSK} and Oka-V. Because Oka-P encodes only Ile at this position, it is likely that the Oka-V_{Merck} passaging history has selected for minor Oka-P-related species that might be present in the Oka-V vaccine. The second substitution (position 107599 in ORF 62 and 122298 in ORF 71) partially changed a Val to Ala in Oka-V_{GSK} and Oka-V; for Oka-V_{Merck}, this position is identical to the one found in Oka-P and encodes Val only. In both cases, the amino acids involved are small hydrophobic residues. Gomi et al. demonstrated that five amino acid substitutions, including the 105356 mutation, in the carboxyl terminus of IE62 directly reduced transactivational activity (22). Experiments with recombinant VZV will be required to determine how these mutations in ORF 62 modulate VZV gene expression and which amino acid substitutions are responsible for the differences in viral spreading.

The product of ORF 10, the virion-associated transactivator, is a tegument protein that regulates the IE62 promoter (28, 46). A nucleotide substitution (C→C/T) at position 12779 results in a conversion of an Ala in the Dumas, Oka-P, and Oka-V_{Merck} strains to a mixture of Ala and Val in the Oka-V and Oka-V_{GSK} strains. Similarly, minor subspecies from Oka-V that were originally present in Oka-P may have been selected in Oka-V_{Merck}. This position, which corresponds to a location in the middle of the protein, in Oka-V and Oka-V_{GSK} encodes two small hydrophobic amino acids (Val and Ala) that could have similar functions. Indeed, no statistically significant differences in transactivational activity of the ORF 10 gene product could be detected between the wild type and the mutant form, suggesting that this alternative form of ORF 10 has a minimal effect on viral attenuation through modulation of the expression level of IE62 (22). Furthermore, in vitro studies have shown that ORF 10 product was dispensable for VZV replication in vitro (10).

The helicase-primase complex. The helicase-primase complex consists of three proteins encoded by ORF 6 (primase) and ORFs 52 and 55 (helicase). Interestingly, we found four amino acid substitutions in these proteins, three of which were described previously (22).

The first amino acid substitution was located near the C terminus of ORF 6 (position 5745, A→G), which was a Ser in Dumas and Oka-P, a Pro in Oka-V and Oka-V_{GSK}, and a

TABLE 6. Comparison of complete genomic sequences of Oka-V_{GSK} and Oka-V_{Merck} vaccine strains of VZV[a]

Feature relative to WT (Dumas)	Position (WT)	Feature in:				Position in:	
		Oka-P	Oka-V	Oka-V_{GSK}	Oka-V_{Merck}	Oka-V_{GSK}	Oka-V_{Merck}
T→T/C	2515	-	X	C	-	2514	2514
A→G (ORF 6), S→P	5745	-	**X**	**X**	A/G	5744	5744
T→C/T (ORF 9A), W→R	10900	-	**X**	**X**	-	10899	10899
C→C/T (ORF 10), A→V	12779	-	**X**	**X**	-	12778	12778
C→C/T (ORF 14), K, silent	20703	-	**-**	X	**-**	20657	20657
C→A (ORF 14), K→N	20787	X	X	C/A	-	20741	20741
A→T (ORF 14), S→T	20879	-	**-**	A/T	**-**	20833	20833
A→G (ORF 18), N, silent	26125	-	**X**	**X**	A/G	26076	26076
T→T/G (ORF 22), P, silent	39227	-	**X**	**X**	-	39178	39178
C→T (R3 repeat), A→V	41485	-	**-**	X	**-**	41436	41436
C→C/T (R3 repeat), A→V	41494	-	**-**	X	**-**	41445	41445
Deletion/insertion	42403	Del AAA	Del AA	Ins A	Del A	42355	42353
A→G (ORF 31), I→V	58595	-	**A/G**	**A/G**	X	58531	58529
Deletion	60278	Del 5A	Del 5A	Del A	Del AA	60214	60211
A→G (ORF 35), A, silent	64067	-	**X**	**X**	A/G	64003	64000
T→C (ORF 39), M→T	71252	-	**X**	**X**	C/T	71188	71185
A→G (ORF 51), T, silent	89734	-	**X**	**X**	-	89659	89656
A→A/G (ORF 52), I→V	90535	-	**X**	**X**	-	90460	90457
T→C (ORF 54), L, silent	94167	-	**X**	**X**	T/C	94092	94089
T→T/C (ORF 55), V→A	97479	-	**-**	**-**	X	97404	97401
T→C/T (ORF 55), C→R	97796	-	**X**	**X**	**-**	97721	97718
T→C (ORF 62), I→V	105356	-	**X**	**X**	T/C	105284	105281
T→C (ORF 62), A, silent	107136	-	**X**	**X**	T/C	107064	107061
A→A/G (ORF 62), V→A	107599	-	**X**	**X**	-	107527	107524
A→A/G	109200	-	**X**	**X**	-	109128	109125
T→C/T	109546	-	**-**	X	**-**	109474	109471
Deletion, AT	110212	-	**X**	**X**	-	110142	110140–110141
T→G	110214	-	**-**	**-**	X	-	110141
Deletion/insertion	112128	Del A	Del A	Ins 5a	Ins A	112064–112068	112063
Deletion/insertion	117769	Del T	Del T	Ins 5T	Ins T	117701–117705	117696
A→C (Ori)	119683	X	-	X	A/C	119617	119608
A→A/G	120351	-	**-**	X	**-**	120287	120278
T→T/C	120697	-	**X**	**X**	-	120633	120624
T→C/T (ORF 71), V→A	122298	-	**X**	**X**	-	122234	122225
A→G (ORF 71), A, silent	122761	-	**X**	**X**	A/G	122697	122688
A→G (ORF 71), I→V	124541	-	**X**	**X**	A/G	124477	124468

[a] WT, wild type. X, difference relative to Dumas strain; -, identical nucleotide relative to Dumas strain; Ori, origin of replication; Del, deletion; Ins, insertion. When applicable, the resulting codon switch is specified. Boldface highlights homologies between genomic sequences of Oka-V and genomic sequences of Oka-V_{GSK} and/or Oka-V_{Merck}.

mixture of both in Oka-V_{Merck}. Pro is a rigid residue that could induce substantial changes in the protein conformation. This nucleotide substitution was also comprised in an AluI restriction site. Interestingly, Quinlivan et al. found no differences between Oka-V_{GSK} and Oka-V_{Merck} by AluI restriction analysis: both Oka-V_{GSK} and Oka-V_{Merck} were A/G (±AluI), whereas Oka-V was G (+AluI) (52).

The second substitution occurred in ORF 52 (position 90535, A→A/G). In this case, the amino acid residue in Oka-P and Oka-V_{Merck} was Ile, which was partially changed to Val in Oka-V and Oka-V_{GSK}.

The last two substitutions were located in ORF 55. Val at position 97479 was partially replaced by Ala in Oka-V_{Merck}, and Cys at position 97796 was partially replaced by Arg in Oka-V and Oka-V_{GSK}.

Gomi et al. demonstrated that pathogenicity and spreading of VZV were affected by mutations in ORFs 6, 10, and 62, whereas ORFs 52 and 55 did not seem to be important for efficient VZV spreading (22). Because these substitutions were only partial, they could result in the coexistence of different helicase-primase activities resulting from different isomeric complexes, as shown by restriction fragment length polymorphism analysis (22). For most of these positions Oka-V_{GSK} was similar to Oka-V, whereas Oka-V_{Merck} was similar to Oka-P.

Envelope glycoproteins. VZV produces at least seven glycoproteins, gK, gC, gB, gH, gL, gI, and gE, which are the products of ORFs 5, 14, 31, 37, 60, 67, and 68, respectively (11). Two putative additional glycoproteins were recently described, gN (ORF 9A) and gM (ORF 50) (55, 77). It is known that VZV glycoproteins induce a strong humoral immune response following either natural infection or vaccination with the Oka strain. The SNPs in the nine VZV glycoproteins were reviewed in a recent comparative analysis (64). Some of them are specific to the vaccine strains and thus could be involved in VZV attenuation.

The product of ORF 68, gE, is the most abundant glycoprotein expressed during infection. A single amino acid substitution in this protein was shown to induce the accelerated replication phenotype of the VZV-MSP mutant strain (57). Recently, Grose at al. reported the sequences of two VZV isolates harboring a D150N mutation within ORF 68 (25). That study identified only one mutation, common to all four Oka

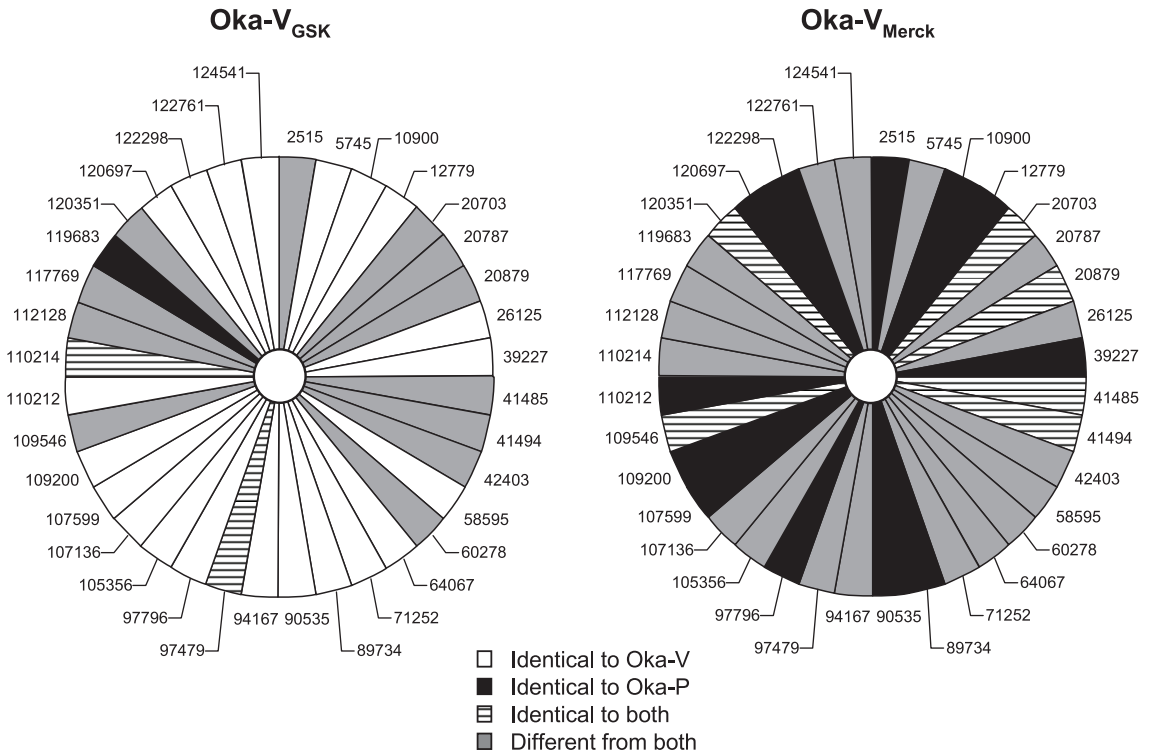

FIG. 3. Sequence comparisons of Oka-V_{GSK} and Oka-V_{Merck} with Oka-P and Oka-V strains of VZV. The 36 nucleotide positions that are different in Oka-V_{GSK} and Oka-V_{Merck} vaccines were compared to the sequence of the original vaccine strain Oka-V and its parental virus, Oka-P.

strains (position 115926, C→T), which induced the replacement of a Thr by Ile.

The product of ORF 31, gB, is the second most abundant and immunogenic envelope glycoprotein of VZV after gE. Along with gH and gC, it seems to play a role in the attachment and penetration of viral particles. It was also shown to have important fusogenic properties in the presence of gE and to be associated with cell-to-cell infection (39, 45). An A→G transition at position 58595 induced a conversion of Ile to Val in Oka-V_{Merck} and a mixture of Ile and Val in both Oka-V_{GSK} and Oka-V. Both amino acids are small and hydrophobic, suggesting that this substitution would probably not affect the properties of this glycoprotein.

ORF 14 exhibited one silent replacement and two amino acid substitutions in its product, gC. At position 20787, a coexistence of C and A induced the partial replacement of a Lys by Asn in Oka-V_{GSK}, whereas the original C residue was completely replaced by A in Oka-V_{Merck}, entirely replacing Lys with Asn. Both residues are hydrophilic; however, Lys is basic and Asn is polar, with an uncharged side chain. Because Asn was not detected in either Oka-P or Oka-V, it is likely that this amino acid substitution evolved as a result of additional vaccine passages and might reflect additional cell culture adaptation. The second modification (position 20879, A→T) was found only in the Oka-V_{GSK} strain. It partially changed a Ser to Thr. Both residues are hydrophilic and polar with an uncharged side chain. Grinfeld et al. showed that products of ORF 14 and ORF 67 were dispensable for the establishment of latency in a rat model (24). However, experiments with SCID-hu mice showed that gC is important for viral tropism in skin cells and that a decrease in gC plays a critical role in attenuation (44). It was also previously shown that expression of gC is dependent on the strain of VZV, with the gC level of Oka-V being much lower than that of wild-type viruses (29, 37).

A nucleotide substitution at position 71252 induced the replacement of a Met by a Thr in the product of ORF 39, one of the two multiply inserted membrane proteins of VZV. This change was complete in Oka-V_{GSK} and Oka-V but only partial in Oka-V_{Merck}. Although both amino acids are neutral, Thr is polar and Met is hydrophobic. This difference may have some effect on the properties of the protein, although these are unclear at present (23).

We also found a mutation in the gN envelope glycoprotein. This glycoprotein is the product of ORF 9A, a newly identified gene positioned closely upstream of the ORF 9 initiation codon (55). Glycoprotein gN is an 87-amino-acid protein whose amino-terminal extremity overlaps with the first nine amino acids of the ORF 8 product, coded on the complementary strand. The observed T-to-T/C shift at position 10900 involves a Trp-to-Arg switch in the very last amino acid of the protein in Oka-V and Oka-V_{GSK}. This change leads to replacement of a hydrophobic amino acid with a large aromatic side chain at the carboxyl terminus of the protein by a hydrophilic basic amino acid, which could alter the membrane topology of the protein or its stability.

R3 repeat region. The R3 reiteration region is located in ORF 22, the longest ORF in VZV. The product of ORF 22 is

TABLE 7. Comparison of Oka-V_{GSK} and Oka-V_{Merck} genomic sequences with previously published Oka genomic sequences[as]

Reference	Position	ORF	Previously reported feature					Feature in present study	
			Dumas	Oka-P	Oka-V	Oka-V_{GSK}	Oka-V_{Merck}	Oka-V_{GSK}	Oka-V_{Merck}
3	84983	48	G	A	A		A	A	A
	85563	48	C	T	T		T	T	T
	86484		cctgataaac		-			cctgataaac	cctgataaac
	86556		T		G			G	G
	87841	50	C		T			T	T
	88477	51	G		T			T	T
	89734	51	A		G			G	A
	89905	51	T		C			C	C
	90202	51	G		T			T	T
	90217	51	T		C			C	C
	90392		G		A			A	A
	91191	52	C		T			T	T
	92092	52	A		G			G	G
	92375	52	A		G			G	G
	92999	52	T		C			C	C
	94167	54	T		C			C	T/C
	94632	54	T		G			G	G
	94641	54	A		T			T	T
	95241	54	T	C	C		C	C	C
	95546	54	G		A			A	A
	97141	55	T		C			C	C
	97470[b]	55	G		C			G	G
	97591	55	C		T			T	T
	97748	55	G		A			A/G	A/G
	97834[c]	55	C		T			C	C
	98437	55	T		C			C	C
	98765	56	T		C			C	C
	98807	56	A		C			C	C
	99227	56	TTC	-	-			TTC	TTC
	99709	58	A	G	G		G	G	G
	99981	58	C	T	T		T	T	T
	100114	58	T	A	A		A	A	A
	100151	58	T	G	G		G	G	G
	100283		A		G			G	G
	101089	59	A	A	G		**A**	A/G	**A/G**
	101331	60	C	T	T		T	T	T
	101623	60	-	+ATC	+ATC			+ATC	+ATC
	101886		T		C			C	C
	101991		C		T			T	T
	102192		G		A			A	A
	102203		A		G			G	G
	102219				+112 bp				
	102309		C		A			A	A
	102351		A		C			C	C
	102458		A		G			G	G
	102601		T		G			G	G
	103043		T		C			C	C
	104898		A		G			G	G
	105010		cctcctct	cctcctct	gcccttacccc		cctcctct	cctcctct	cctcctct
	105054		G		Del G			Del G	Del G
	105063[d]		G		Del G			Del G	Del G
	105145			+AACA	+AACA			+AACA	+ACAA
	105310	62	A	A	G		**G**	A/G	**A/G**
	105312	62	A	G	G		G	G	G
	105356	62	T	T	C		C	C	C
	105451	62	A		G			G	G
	105512	62	A		C			C	C
	105544	62	A		G			G	G
	105705	62	T		C			C	C
	106262	62	T	T	C		C	C	C
	107136	62	T		C			C	C
	107165	62	C		T			T	T
	107252	62	T		C			C	C
	107307	62	T		C			C	C
	107607	62	C		A			A	A
	107715	62	T		C			C	C

Continued on following page

TABLE 7—*Continued*

Reference	Position	ORF	Previously reported feature					Feature in present study	
			Dumas	Oka-P	Oka-V	Oka-V_{GSK}	Oka-V_{Merck}	Oka-V_{GSK}	Oka-V_{Merck}
	108111	62	T		C			C	C
	108747	62	A		G			G	G
	108951	62	G		A			A	A
	109044	62	C		G			G	G
	109694[e]				+ATC			+CAT	+CAT
	109762				+27 bp			C	C
	110196				Del TA			No del	No del
	110216		$(ga)_9gg$		$(ta)_6(gc)_2aaga$		**$(ta)_{16}gag(ga)_4$**		**$(ta)_{10}(ga)_9aaa(ga)_4$**
	110378				Del GC			Del GC	Del GC
	111312	63	A		G			G	G
	111650	64	A	A	G		A/G	A/G	A/G
	112093	64	T	C	C		C	C	C
	112130[f]		-	-	$+A_8$		**$+A_8$**	$+A_{12}$	**$+A_9$**
	112198		G	A	A		A	A	A
	114140	66	A		G			G	G
	115041	67	G		A			A	A
	115926	68	C		T			T	T
60	1		A	G	G	G	**A**	G	**G**
	3		G	C	C	C	**G**	C	**C**
	178		G	G	G	C	C	C	C
	560		T	T	C	C	C	C	C
	703[g]	1	T	T	T	C	C	C	C
	82225[h]	45	A	A	A	**G**	A	**A**	A
	86363	49	A	A	A	**T**	A	**A**	A
	87677	50	A	A	A	**G**	A	**A**	A
	89734	51	A	A	G	**A**	A	**G**	A
	90115[i]	51	A	A	stop	A	A	A	A
	105054		G	-	-	-	**G**	Del G	**Del G**
	105071		G	G	-	-	**G**	Del G	**Del G**
	105145	Poly(A)	-	AACA	AACA	**AACA**	-	**ACAA**	**ACAA**
	105169		A	A	A/G	A/G	**A**	A/G	**A/G**
	105310	62	A	A	A/G	**G**	**A**	**A/G**	**A/G**
	105356	62	T	T	C	C	**T**	C	**T/C**
	105544	62	A	A	G	G	G	G	G
	124353	71	T	T	C	C	C	C	C
	124541	71	A	A	G	G	**G**	G	**A/G**
	124587	71	T	T	C/T	C	**T**	C	**C/T**
	124728		T	T	C/T	C/T	**T**	C/T	**C/T**
	124750	pA-71	-	TGTT	TGTT	TGTT	TGTT	TGTT	TGTT
	124834		C	-	-	-	**C**	Del C	**Del C**
	124851		C	-	-	-	**C**	Del C	**Del C**
22	560			T	**C**			**C**	**C**
	703	1		T	T/C			C	C
	763	1		T	T/C			C	C
	2515			T	T/C			C	T
	5745	6		A	**G**			**G**	A/G
	10900	9A		T	**T/C**			**T/C**	T
	12779[j]	10		T	**T/C**			**T/C**	C
	19431	14		T	T/C			T	T
	26125	18		A	**G**			**G**	A/G
	31732	21		C	T/C			C	C
	38036[k]	22		T	**T/C**			**T/C**	**T/C**
	39227	22		T	**T/G**			**T/G**	T
	58595	31		A	**A/G**			**A/G**	G
	59287	31		A	**A/G**			**A/G**	**A/G**
	64067[l]	35		A	A/G			G	A/G
	71252[m]	39		T	T/C			C	T/C
	82225	45		A	A/G			A	A
	84091	47		G	A/G			G	G
	87280	50		A	A/G			A	A
	87306	50		T	T/C			T	T
	89734[n]	51		A	A/G			G	A
	90535	52		A	**A/G**			**A/G**	A

Continued on following page

TABLE 7—*Continued*

Reference	Position	ORF	Previously reported feature					Feature in present study	
			Dumas	Oka-P	Oka-V	Oka-V_{GSK}	Oka-V_{Merck}	Oka-V_{GSK}	Oka-V_{Merck}
	94167	54		T	**C**			**C**	T/C
	97748	55		G	**A/G**			**A/G**	**A/G**
	97796	55		T	**T/C**			**T/C**	T
	101089[o]	59		A	**A/G**			**A/G**	**A/G**
	105169			A	**A/G**			**A/G**	**A/G**
	105310	62		A	**A/G**			**A/G**	**A/G**
	105356	62		T	**C**			**C**	T/C
	105544	62		A	**G**			**G**	**G**
	105705	62		T	**C**			**C**	**C**
	106262	62		T	**C**			**C**	**C**
	106710[p]	62		A	A/G			A	A
	107136	62		T	**C**			**C**	T/C
	107252	62		T	**C**			**C**	**C**
	107599	62		A	**A/G**			**A/G**	A
	107797[q]	62		A	A/G			A	A
	108111	62		T	**C**			**C**	**C**
	108838	62		A	**A/G**			**A/G**	**A/G**
	109137			A	**A/G**			**A/G**	**A/G**
	109200			A	**A/G**			**A/G**	A
	111650[r]	64		A	A/G			A/G	A/G

[a] Boldface highlights differences (3, 60) and homologies (22) between results from published studies and results from the present study. Lowercase indicates insertion; -, missing nucleotide position.
[b] Indicated as G in the Oka-V GenBank submission AB097932.
[c] Indicated as C in the Oka-V GenBank submission AB097932.
[d] In the present alignment, this position is 105071.
[e] In the present alignment, this position is 109696.
[f] In the present alignment, this position is 112128.
[g] Indicated as Y in the Oka-V GenBank submission AB097932.
[h] Indicated as R in the Oka-V GenBank submission AB097932.
[i] Indicated as A in the Oka-V GenBank submission AB097932.
[j] Indicated as C in the Oka-P GenBank submission AB097933.
[k] Indicated as T in the Oka-V GenBank submission AB097932.
[l] Indicated as G in the Oka-V GenBank submission AB097932.
[m] Indicated as C in the Oka-V GenBank submission AB097932.
[n] Indicated as G in the Oka-V GenBank submission AB097932.
[o] Indicated as R in the Oka-P GenBank submission AB097933.
[p] Indicated as A in the Oka-V GenBank submission AB097932.
[q] Indicated as A in the Oka-V GenBank submission AB097932.
[r] Indicated as A in the Oka-V GenBank submission AB097932.

homologous to the UL36 virion tegument phosphoprotein of herpes simplex virus type 1 (41, 42). R3 is a highly variable region consisting of repeated elements that can vary in number and combination (12, 22), but the impact of this region on the function of ORF 22 phosphoprotein remains mostly unknown because the function of the phosphoprotein itself is poorly understood.

Despite the high variability of the R3 region, no frameshift mutations were detected in ORF 22, because the repeated elements are present as multiples of 3 bp. Nevertheless, two mutations (positions 41485 and 41494, C→T) in the Oka-V_{GSK} strain were identified, both of which convert Ala into Val, another amino acid with similar properties that probably does not affect the function of the ORF 22 protein.

Attenuation and reactivation. Comparison of the four Oka strain sequences of VZV indicated that Oka-V_{GSK} is genetically closer to Oka-V than Oka-V_{Merck} is but that Oka-V_{Merck} is closer to Oka-P than Oka-V_{GSK} is (reference 50 and the present study). In agreement with these findings, Sauerbrei et al. demonstrated that Oka-V_{Merck} is genetically closer to Oka-P than Oka-V_{GSK} is (59). Nevertheless, it is well established that both vaccine strains are attenuated but remain strongly immunogenic (43, 73). Recently, Quinlivan et al. suggested an association of particular SNPs in the VZV genome with frequency of vaccine-induced rash (53). Four SNP positions were suggested to contain nucleotides specific to Oka-P in most of the viruses isolated from vaccine rashes. The first one is a silent nucleotide change within ORF 51 (A→G at position 89734). The nucleotide at this position is A in Oka-V_{Merck} and Oka-P but G in Oka-V_{GSK} and Oka-V. The second SNP, at position 105169, contains mixed A/G nucleotides for Oka-$V_{Merck,}$ Oka-$V_{GSK,}$ and Oka-V, whereas the parental Oka-P contains only A. The third SNP, at position 105356, is located within ORF 62. The change from T to C is responsible for an amino acid switch (Ile→Val) for both Oka-V_{GSK} and Oka-V. At the same position, the parental Oka-P contains T (Ile), and Oka-V_{Merck} contains a mixture of T and C (Ile/Val). The last position (nucleotide 107797) was not identified as a SNP in our sequencing data, which agrees with a previous study (22).

Although the genetic basis of Oka-V attenuation has not been determined, Oka-V and Oka-P genomes have nucleotide differences predicted to change amino acids in every class of viral proteins (3, 21). VZV attenuation is a multifactorial phe-

nomenon whose mechanism remains unclear (80), but it is conceivable that mutations of the vaccine genome, in particular, mutations resulting in amino acid modifications, could affect virulence or latency of the vaccine strain. Recently, Peters and coworkers sequenced 11 VZV genomes from different clades, bringing the current number of available full-length VZV sequences to 18 (50, 69). To assess variations that can occur during serial passage in cell culture, these studies included the four Oka strains (Oka-P and the three Oka vaccine strains) and a VZV strain sequenced at passages 5, 22, and 72. As discussed by Tyler et al. (69), the SNPs in ORF 62/71 found in the three Oka vaccine strains and in the VZV strain at high passage level (S628G, R958G, and I1260V in IE62) could be involved in the attenuation of VZV. In addition, it was suggested that other mutations could play a role, particularly those in regions containing ORFs 30 to 55 (69, 78, 80). However, we found that numerous SNPs contain the original Oka-P nucleotide, supporting the idea that the attenuated phenotype is the result of a cooperative effect between several SNPs rather than the result of selected mutations. Therefore, our analysis of SNP importance is aligned with the conclusions of Tyler et al. regarding VZV attenuation (69).

VZV remains latent in sensory-nerve ganglia and can reactivate later, causing herpes zoster. It was suggested that herpes zoster is less common after vaccination, because initial access of Oka-V to neural cells is reduced by limited skin replication or because Oka-V reactivation and secondary viral infection of skin are less efficient, rather than because of an intrinsic attenuation of Oka-V neurotropism (5). Indeed, even though the Oka-V strain of VZV can cause herpes zoster, it seems to reactivate less often than the wild-type VZV even in immunocompromised children (18, 27, 68), and increased incidences of reactivation after vaccination have not been demonstrated in either clinical studies or postmarketing surveillance (33a, 43, 62, 66, 70, 73). In addition, the Oka vaccines have been shown to elicit a strong and protective immune response against varicella (7, 43, 73).

Conclusion. Overall, this study shows that, throughout the entire VZV genome, only 36 nucleotide positions differ between the Oka-V_{GSK} and Oka-V_{Merck} vaccine strains. Analysis of the complete genome of VZV also shows that, genetically, Oka-V_{GSK} is closer to Oka-V and that Oka-V_{Merck} is closer to Oka-P. Although Oka-V_{GSK} and Oka-V_{Merck} exhibit differences, there is a high degree of conservation between these strains at both the nucleotide and amino acid levels. This result supports the clinical data showing that both vaccines are well tolerated and elicit strong immune responses against varicella.

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