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(54) **VARIANT VARICELLA-ZOSTER VIRUSES AND METHODS OF USE**

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(51) **Int. Cl.**[7] **A51K 39/245**
(52) **U.S. Cl.** **424/230.1**; 424/204.1; 424/130.1; 435/6; 435/975; 435/345
(58) **Field of Search** 424/230.1, 204.1, 424/130.1; 435/6, 975, 345

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,985,615	A	10/1976	Kubo
5,462,734	A	10/1995	Letchworth, III et al.
5,595,890	A	1/1997	Newton et al.
5,710,248	A	1/1998	Grose et al.
5,849,476	A	12/1998	Shiraki et al.
5,952,174	A	9/1999	Nikiforov et al.
6,087,170	A	7/2000	Kemble

FOREIGN PATENT DOCUMENTS

WO WO 92/06989 * 4/1992

OTHER PUBLICATIONS

Valcarcel et al, Vaccine, 1997, Vol. 15, No. 6/7, pp. 709–719.*

Wu et al., Archives of Virology, 1997, Vol 142, pp. 349–362.*

Fowler et al, Virology, 1995, Vol. 214, pp. 531–540.*

A. M. Arvin et al., "Live Attenuated Varicella Vaccine," *Annu. Rev. Microbiol., 50,* 59–100 (1996).

A. M. Arvin et al., "Immunity to Varicella–Zoster Viral Glycoproteins, gp I (gp 90/58) and gp III (gp 118), and to a Nonglycosylated Protein, p. 170," *J. Immunol. 137,* 1346–1351 (1986).

ATCC CCL–171, "*Homo sapiens* (human)," [online]. Retreived on May 16, 2001. Retrieved from the Internet; <URL:http://phage.atcc.org/cgi–bin/searchengine/longview.cgi!view=ce, 317407, CCL–171&ctext=CCL.–1,3 pages.

ATCC VR–586, "Variella–Zoster," [online]. Retrieved on Jun. 5, 2001. Retrived from the Internet <URL:http//phage.atcc.org/cgi–bin/seachengine/longview.cgi!view=av, 343894, VR–586&text=VR–586>, 2 pages.

ATCC VR–795, "Varicella–Zoster deposited as Varicella," [online]. Retrieved on May 16, 2001. Retrieved from the Internet:<URL:http://phage.atcc.org/cgi–bin/searchengine/longview.cgi!view=av,476976, VR–795&text=VR–7.9>, 2 pages.

ATCC VR–916, "Varicella–Zoster deposited as Varicella," [online]. Retrieved on Sep. 6, 2000. Retrieved from the Internet:<URL:http//phage.atcc.org/cgi–bin/searchengine/longview.cgi!view=av,554286&text=varicella>, 1 page.

ATCC VR–1367, "Varicella–Zoster," [online]. Retrieved on Jun. 5, 2001. Retrieved from the Internet:<URL:http://phage.atcc.org/cgi–bin/searchengine/longview.cgi!view=av, 871705, VR–1367&text=VR–1367>, 2 pages.

M. F. Bachman et al., "The influence of Antigen Organization on B Cell Responsiveness," *Science, 262,* 1448–1451 (1993).

R. E. Bergen et al., "Human T Cells Recognize Multiple Epitopes of an Immediate Early/Tegument Protein (IE62) and Glycoprotein I of Varicella Zoster Virus," *Viral Immunol., 4,* 151–166 (1991).

J. I. Cohen et al., "Generations of varicella–zoster virus (VZV) and viral mutants from cosmid DNAs: VZV thymidylate synthetase is not essential for replication in vitro," *Proc. Natl. Acad. Sci. USA, 90,* 7376–7380 (1993).

N. L. Cole et al., "Colchicine treatment in the preparation of varicella–zoster virus inocula," *J. Virol. Methods, 36,* 111–118 (1992).

A. J. Davison et al., "The Complete DNA Sequence of Varicella–Zoster Virus," *J. Gen. Virol., 67,* 1759–1816 (1986).

K. S. Dingwell et al., "Herpes simplex virus glycoproteins E and I facilitate cell–to–cell spread in vivo across junctions of cultured cells," *J. Virol., 68* 834–845 (1994).

K. M. Duus et al., "Cell Surface Expression and Fusion by the Varicella–Zoster Virus gH:gL Glycoprotein Complex: Analysis by Laser Scanning Confocal Microscopy," *Virology, 210,* 429–440 (1995).

(List continued on next page.)

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(57) **ABSTRACT**

The present invention provides methods directed to detecting antibodies that specifically bind to a varicella zoster polypeptide, detecting the presence of a varicella zoster virus in an animal, diagnosing a disease caused by varicella zoster virus, and detecting a varicella zoster virus having a single nucleotide polymorphism in ORF68. The present invention also provides a vaccine composition, a method for producing a modified attenuated varicella zoster virus, isolated polynucleotides, and isolated polypeptides, and viruses.

8 Claims, 10 Drawing Sheets

OTHER PUBLICATIONS

K. M. Duus et al., "Multiple Regulatory Effects of Varicella–Zoster Virus (VZV) gL on Trafficking Patterns and Fusogenic Properties of VZV gH," *J. Virol., 70,* 8961–8971 (1996).

J. R. Ecker et al., "Varicella zoster virus DNA exists as two isomers," *Proc. Natl. Acad. Sci. USA, 79,* 156–160 (1982).

T. Gojobori et al., "Rates of evolution of the retroviral oncogene of Maloney murine sarcoma virus and of its cellular homologues," *Proc. Natl. Acad. Sci. USA, 82,* 4198–4201 (1985).

F. L. Graham et al., "Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5," *J. Gen. Virol., 36,* 59–74 (1977).

C. Grose et al., "Computer modeling of prototypic and aberrant nucleocapsids of varicella–zoster virus," *Virology, 214,* 321–329 (1995).

C. Grose, "Glycoproteins Encoded by Varicella–Zoster Virus: Biosynthesis, Phosphorylation, and Intracellular Trafficking," *Annu. Rev. Microbiol., 44,* 59–80 (1990).

C. Grose, "Pathogenesis of Infection with Varicella Vaccine," *Infect. Dis. Clinics NA, 10,* 489–505 (1996).

C. Grose, et al., "*Varicella–Zoster Virus: Isolation and Propagation in Human Melanoma Cells at 36 and 32°* C.," *Infect. Immun. 19,* 199–203 (1978).

C. Grose, et al., "Monoclonal Antibodies Against Three Major Glycoproteins of Varicella–Zoster Virus," *Infect. Immun., 40,* 381–388 (1983).

C. Grose, "The Synthesis of Glycoproteins in Human Melanoma Cells Infected with Varicella–Zoster Virus," *Virology, 101*:1–9 (1980).

B. H. Hahn et al., "Genetic variation in HTLV–III/LAV over time in patients with AIDS or at risk for AIDS," *Science, 232,* 1548–1553 (1986).

R. Harson et al., "Egress of Varicella–Zoster Virus from the Melanoma Cell: a Tropism for the Melanocyte," *J. Virol., 69,* 4994–5010 (1995).

C. Hatfield et al., "Epitope Mapping and Tagging by Recombination PCR Mutagenesis," *Bio Techniques, 22,* 332–337 (1997).

J. Holland et al., "Rapid evolution of RNA genomes," *Science, 215,* 1577–1585 (1982).

M. Ito et al., "Human Leukocytes Kill Varicella–Zoster Virus–Infected Fibroblasts in the Presence of Murine Monoclonal Antibodies to Virus–Specific Glycoproteins," *J. Virol., 54,* 98–103 (1985).

D. H. Jones et al., "A Rapid Method for Site–Specific Mutagenesis and Directional Subcloning by Using the Polymerase Chain Reaction to Generate Recombinant Circles," *Bio Techniques, 8,* 178–183 (1990).

F. Jones et al., "Role of cytoplasmic vacuoles in varicella–zoster virus glycoprotein trafficking and virion envelopment," *J. Virol., 62,* 2701–2711 (1988).

P. R. Kinchington et al., "Regulated Nuclear Localization of the Varicella–Zoster Virus Major Regulatory Protein, IE62," *J. Infect. Dis., 178*(*Suppl. 1*), S16–21 (1998).

P. R. Kinchington et al., "Molecular basis for a geographic variation of varicella–zoster virus recognized by a peptide antibody," *Neurology, 45* (*Suppl 8*), S13–14 (1995).

P. LaRussa et al., "Restriction fragment length polymorphism of polymerase chain reaction products from vaccine and wild–type varicella–zoster virus isolates," *J. Virol. 66,* 1016–1020 (1922).

V. Litwin et al., "Receptor Properties of Two Varicella–Zoster Virus Glycoproteins, gpI and gpIV, Homologous to Herpes Simplex Virus gE and gI," *J. Virol., 66,* 3643–3651 (1992).

V. Litwin et al., "Cell Surface Expression of the Varicella–Zoster Virus Glycoproteins and Fc Receptor," *Virology, 178,* 263–272 (1990).

S. Mallory et al., "Mutational Analysis of the Role of Glycoprotein I in Varicella–Zoster Virus Replication and Its Effect on Glycoprotein E Conformation and Trafficking," *J. Virol., 71,* 8279–8288 (1997).

D. J. McGeoch et al., "Molecular phylogeny of the alphaherpesvirinae subfamily and a proposed evolutionary timescale," *J. Mol. Biol., 238,* 9–22 (1994).

D. J. McGeoch et al., "Molecular phylogeny and evolutionary timescale for the family of mammalian herpesviruses," *J. Mol. Biol., 247,* 443–458 (1995).

J. F. Moffat et al., "Tropism of Varicella–Zoster Virus for Human $CD4^+$and $CD8^+$T Lymphocytes and Epidermal Cells in SCID–hu Mice," *J. Virol., 69,* 5236–5242 (1995).

J. F. Moffat et al., "Attenuation of the Vaccine Oka Strain of Varicella–Zoster Virus and Role of Glycoprotein C in Alphaherpesvirus Virulence Demonstrated in the SCID–hu Mouse," *J. Virol., 72,* 965–974 (1998).

E. A. Montalvo et al., "Assembly and Processing of the Disulfide–Linked Varicella–Zoster Virus Glycoprotein gpII(140)," *J. Virol., 61,* 2877–2884 (1987).

National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Genbank X04370. Accession Number X04370 M14891 M16612, "The complete DNA sequence of varicella–zoster virus," [online]. J. Gen. Virol., 67 (Pt 9) 1759–1816 (1986), [retrieved on May 29, 2001]. Retrieved from the Internet:<URL: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi!cmd=Retrieve&db=Nucleotide&list uids=599.8=GenBank>, 39 pages.

T. I. Ng et al., "Phosphorylation of Varicella–Zoster Virus Open Reading Frame (ORF) 62 Regulatory Product by Viral ORF 47–Associated Protein Kinase," *J. Virol., 68,* 1350–1359 (1994).

N. Nishimura et al., "A Di–Acidic Signal Required for Selective Export from the Endoplasmic Reticulum", *Science, 277,* 556–560 (1997).

J. K. Olson et al., "Complex formation facilitates endocytosis of varicella–zoster virus gE:gI Fc receptor," *J. Virol., 72,* 1542–1551 (1998).

J. K. Olson et al., "Varicella–Zoster Virus Fc Receptor gE Glycoprotein: Serine/Threonine and Tyrosine Phosphorylation of Monomeric and Dimeric Forms," *J. Virol., 71,* 110–119 (1997).

J. K. Olson et al., "Endocytosis and Recycling of Varicella–Zoster Virus Fc Receptor Glycoprotein gE: Internalization Mediated by a YXXL Motif in the Cytoplasmic Tail," *J. Virol., 71,* 4042–4054 (1992).

J. A. Padilla et al, "High–resolution immuno–scanning electron microscopy using a non–coating method: study of herpes simplex virus glycoproteins on the surface of virus particles and infected cells," *J. Elect. Microscopy, 46,* 171–180 (1997).

Public Law 94–279; Apr. 22, 1976, United States Statutes at Large, vol. 90.

B. Rentier, "Introduction", *Neurol, 45(Suppl. 8)*, S8 (1995).
J. E. Rodriguez et al., "Entry and egress of varicella virus blocked by same anti–gH monoclonal antibody," *Virology, 196,* 840–844 (1993).
J. Sambrook et al, *Molecular Cloning: A Laboratory Manual., Second Edition,* Cold Spring Harbor Laboratory Press (1989), (Cover Page, publication page and table of contents).
R. A. Santos et al., "Varicella–Zoster Virus gE Escape Mutant VZV–MSP Exhibits an Accelerated Cell–to–Cell Spread Phenotype in both Infected Cell Cultures and SCID–hu Mice," Virology, 275, 306–317 (September 2000).
R. A. Santos et al., "Antigenic Variation of Varicella Zoster Virus Fc Receptor gE: Loss of a Major B Cell Epitope in the Ectodomain," *Virology, 249,* 21–31 (1998).
T. Shioda et al., "Small amino acid changes in V3 hypervariable region of gp120 can affect the T–cell–line and macrophage tropsim of human immunodeficiency virus type 1," *Proc. Natl. Acad. Sci. USA, 89,* 9434–9438 (1992).
F. I. Smith et al., "Variation in influenza virus genes epidemiological, pathogenic, and evolutionary consequences", *The Influenza Virues,* (R.M. Krug, Ed.), 319–359. Plenum Press, New York (1989).
P. G. Spear, "Glycoproteins Specified by Herpes Simplex Viruses," *The Herpesviruses, 3,* (B. Roizman, Ed.), 315–356. Plenum Press, New York (1985).
E. Szomolanyi–Tsuda et al., "T cell–independent antibody–mediated clearance of polyomo virus in T cell–deficient mice," *J. Exp. Med., 183,* 403–411 (1996).
M. Takahashi et al., "Development of a Live Attenuated Varicella Vaccine," *Biken, J., 18,* 25–33 (1975).
T. A. Tatusova, et al., "BLAST 2 SEQUENCES, a new tool for comparing protein and nucleotide sequences," *FEMS Microbiol Lett, 174,* 247–250 (1999), and available at http://www.ncbi.nlm.nih.gov/gorf/bl2.html.
U.S. National Institutes of Health, "About NIH Image," [online]. Retreived on May 29, 2001. Retrieved from the Internet:<URL:http//rsb.info.nih.gov/nih–image/about.html, 2 pages.
K. A. Weigle et al., "Common expression of varicella–zoster viral glycoprotein antigens in vitro and in chickenpox and zoster vesicles," *J. Infect. Dis., 148,* 630–638 (1983).
T. H. Weller, "Serial propagation in vitro of agents producing inclusion bodies derived from varicella and herpes zoster," *Proc. Soc. Exp. Biol. Med., 83,* 340–346 (1953).
S. A. Whaton et al., "Structure function, and antigenicity of the hemagglutinin of influenza viruses," *The Influenza Viruses,* (R. M. Krug, Ed.), 153–171. Plenum Press, New York, (1989).
M. Yang et al., "Retrograde Transneuronal Spread of Pseudorabies Virus in Defined Neuronal Circuitry of the Rat Brain is Facilitated by gE Mutations that Reduce Virulence," *J. Virol., 73,* 4350–4359 (May, 1999).
Z. Yao et al., "Varicella–Zoster Virus Glycoprotein gpI/gpIV Receptor: Expression, Complex Formation, and Antigenicity within the Vaccinia Virus–T7 RNA polymerase Transfection System," *J. Virol., 67,* 305–314 (1993).
Z. Zhu et al, "Targeting of glycoprotein I (gE) of varicella–zoster virus to the trans–Golgi network by an AYRV sequence and an acidic amino acid–rich patch in the cytosolic domain of the molecule," *J. Virol., 70,* 6563–6575 (1996).

* cited by examiner

Fig. 1A

(SEQ ID NO:72)

```
  1  MGTVNKPVVG VLMGFGIITG TLRITNPVRA SVLRYDDFHT DEDKLDTNSV
 51  YEPYYHSDHA ESSWVNRGES SRKAYDHNSP YIWPRNDYDG FLENAHEHHG
101  VYNQGRGIDS GERLMQPTQM SAQEDLGDDT GIHVIPTLNG DDRHKIVNVD
151  QRQYGDVFKG DLNPKPQGQR LIEVSVEENH PFTLRAPIQR IYGVRYTETW
201  SFLPSLTCTG DAAPAIQHIC LKHTTCFQDV VVDVDCAENT KEDQLAEISY
251  RFQGKKEADQ PWIVVNTSTL FDELELDPPE IEPGVLKVLR TEKQYLGVYI
301  WNMRGSDGTS TYATFLVTWK GDEKTRNPTP AVTPQPRGAE FHMWNYHSHV
```

Fig. 1B

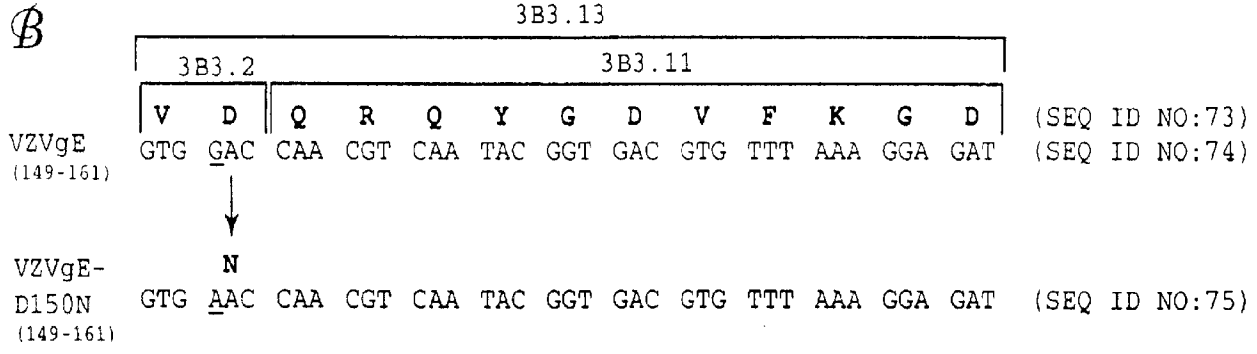
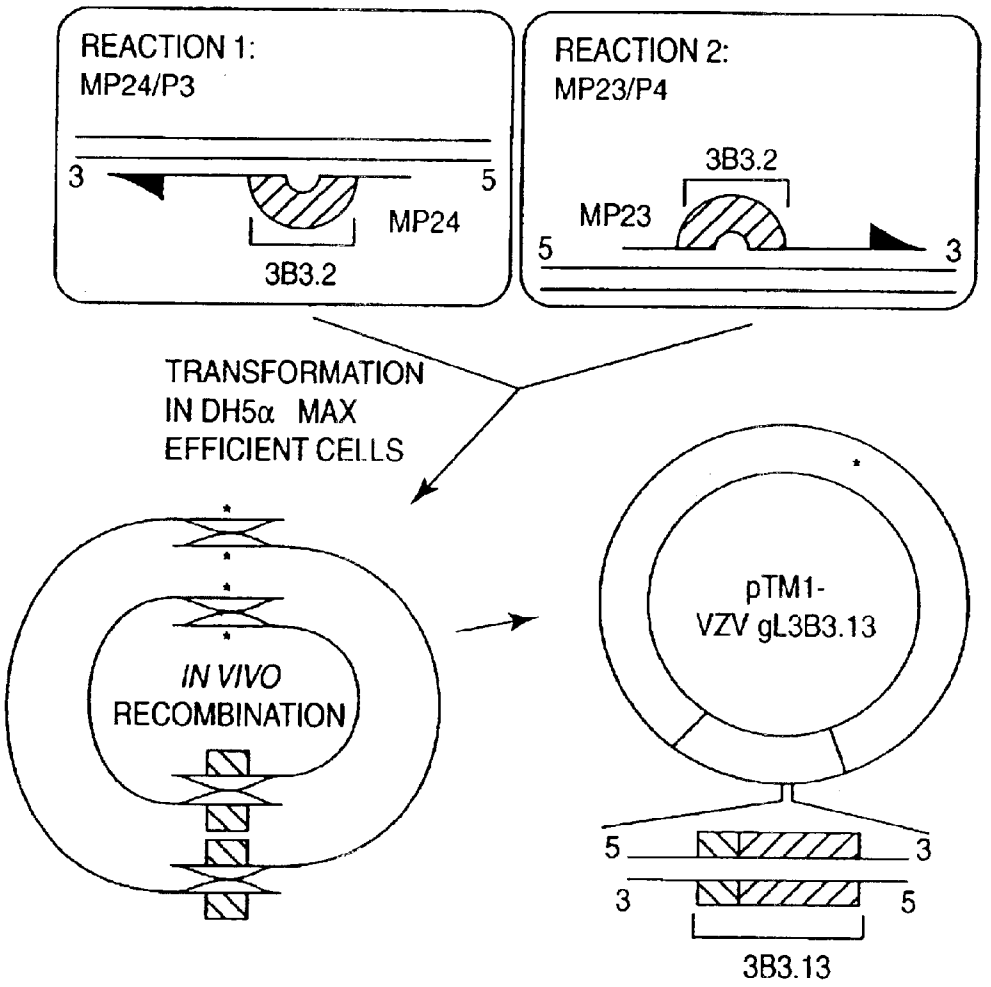


Fig. 2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MP24/P3
3
5
MP24
3B3.2
REACTION 2:
MP23/P4
3B3.2
MP23
5
3
TRANSFORMATION
IN DH5α MAX
EFFICIENT CELLS
IN VIVO
RECOMBINATION
pTM1-
VZV gL3B3.13
5
3
3
5
3B3.13

Fig. 3

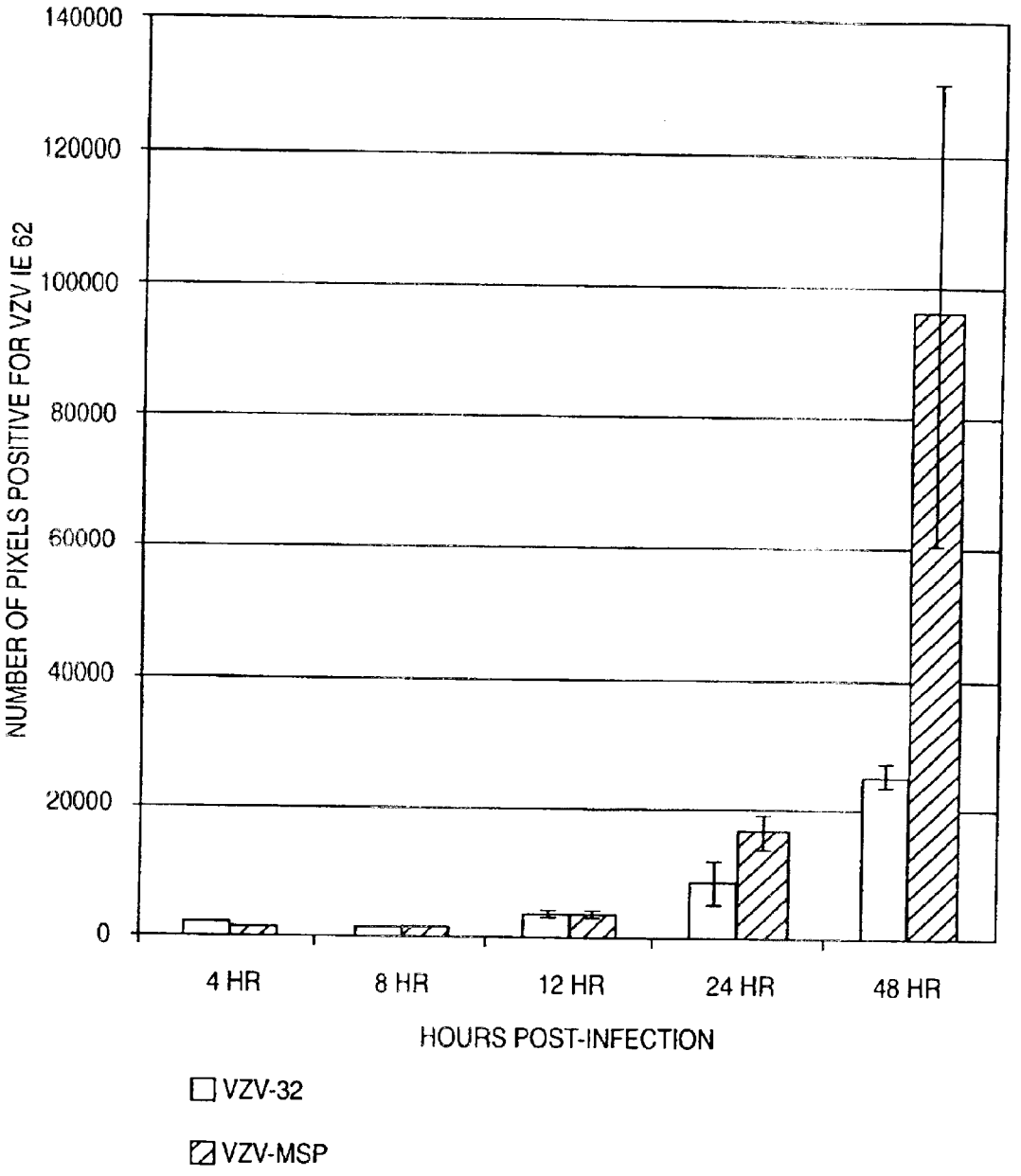

140000
120000
100000
80000
60000
40000
20000
0
NUMBER OF PIXELS POSITIVE FOR VZV IE 62
4 HR
8 HR
12 HR
24 HR
48 HR
HOURS POST-INFECTION
VZV-32
VZV-MSP

Fig. 4

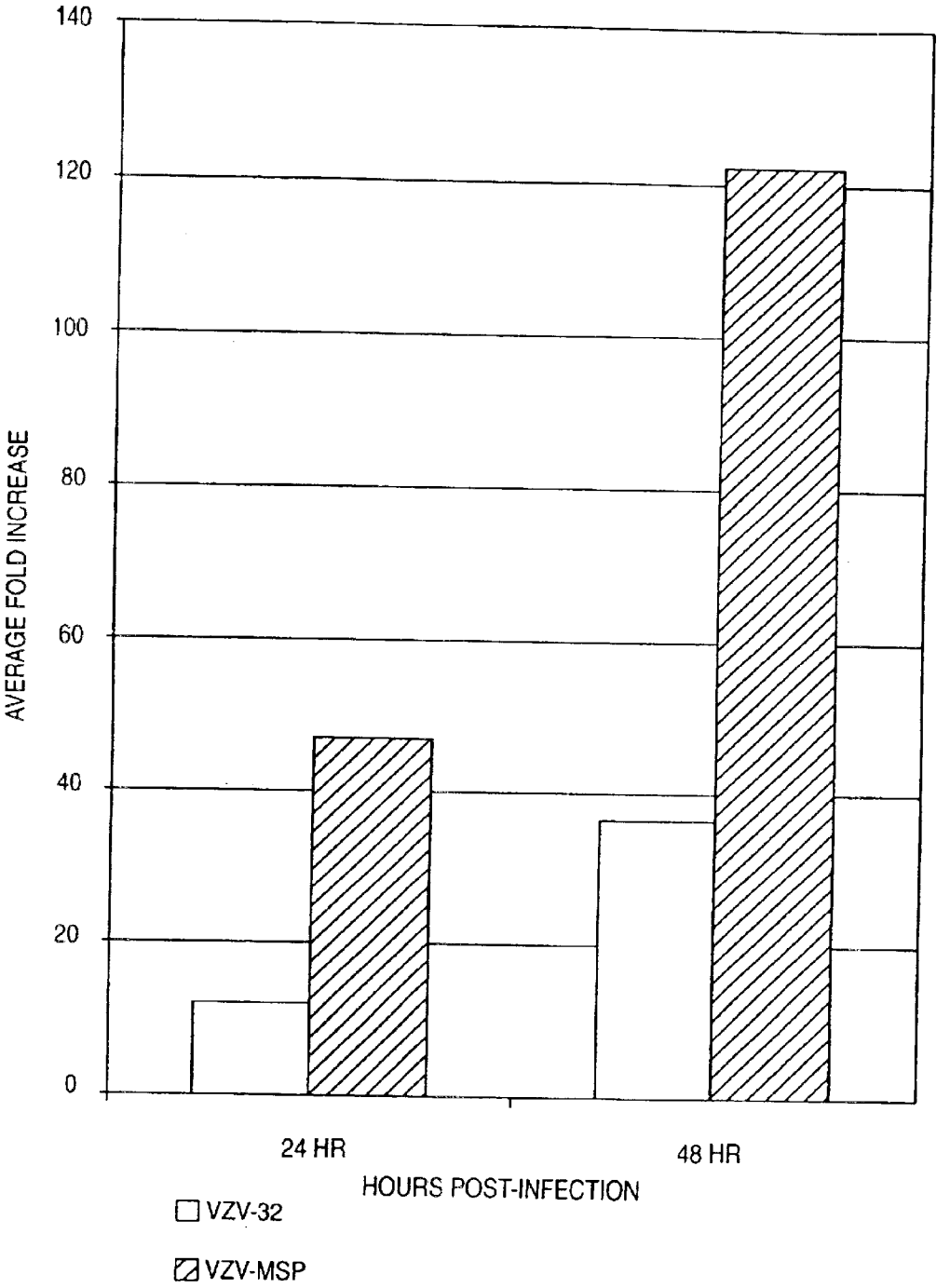
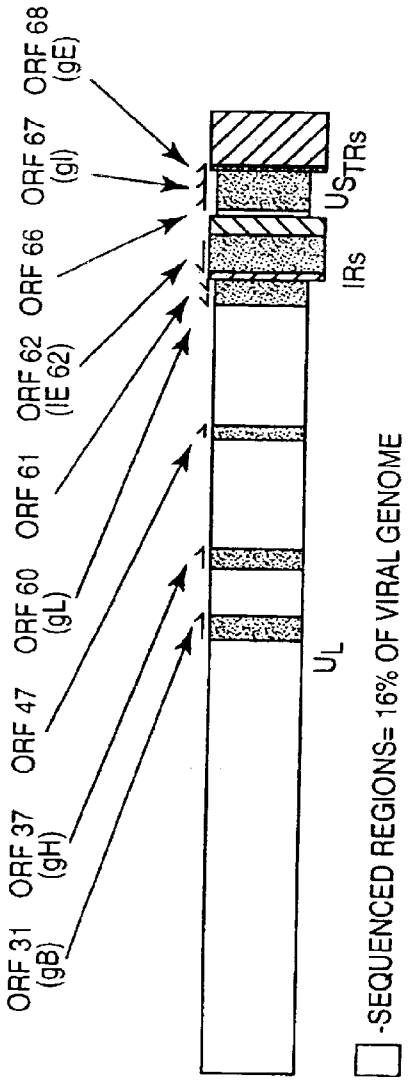

140
120
100
80
60
40
20
0
AVERAGE FOLD INCREASE
24 HR
48 HR
HOURS POST-INFECTION
VZV-32
VZV-MSP

Fig. 5



NUCLEOTIDES	SIZE OF REGION	ORF(s)	SUBSTITUTIONS
56767-57007	241	5' OF ORF 31 (gB)	NONE
57008-59614	2607	ORF 31 (gB)	NONE
66074-68599	2526	ORF 37 (gH)	c66879t(P269L)
83168-84697	1530	ORF 47	NONE
101170-101649	480	ORF 60 (gL)	NONE
101650-103081	1432	5' OF ORF 60 (gL)	a102203g
			a102575g
			c102617t
			a102969g
103082-104485	1404	ORF 61	NONE
104486-104936	450	5' OF ORF 61	a104898g
105201-109133	3933	ORF 62 (IE62)	c109044g (A90A)
109134-109659	525	5' OF ORF 62	NONE
113037-114218	1182	ORF 66	a114140g (S368S)
114219-114495	277	5' of ORF 67 (gI)	NONE
114496-115560	1065	ORF 67 (gI)	NONE
115561-115807	247	5' OF ORF 68 (gE)	NONE
115808-117679	1872	ORF 68 (gE)	g116255a (D150N)

Fig. 6

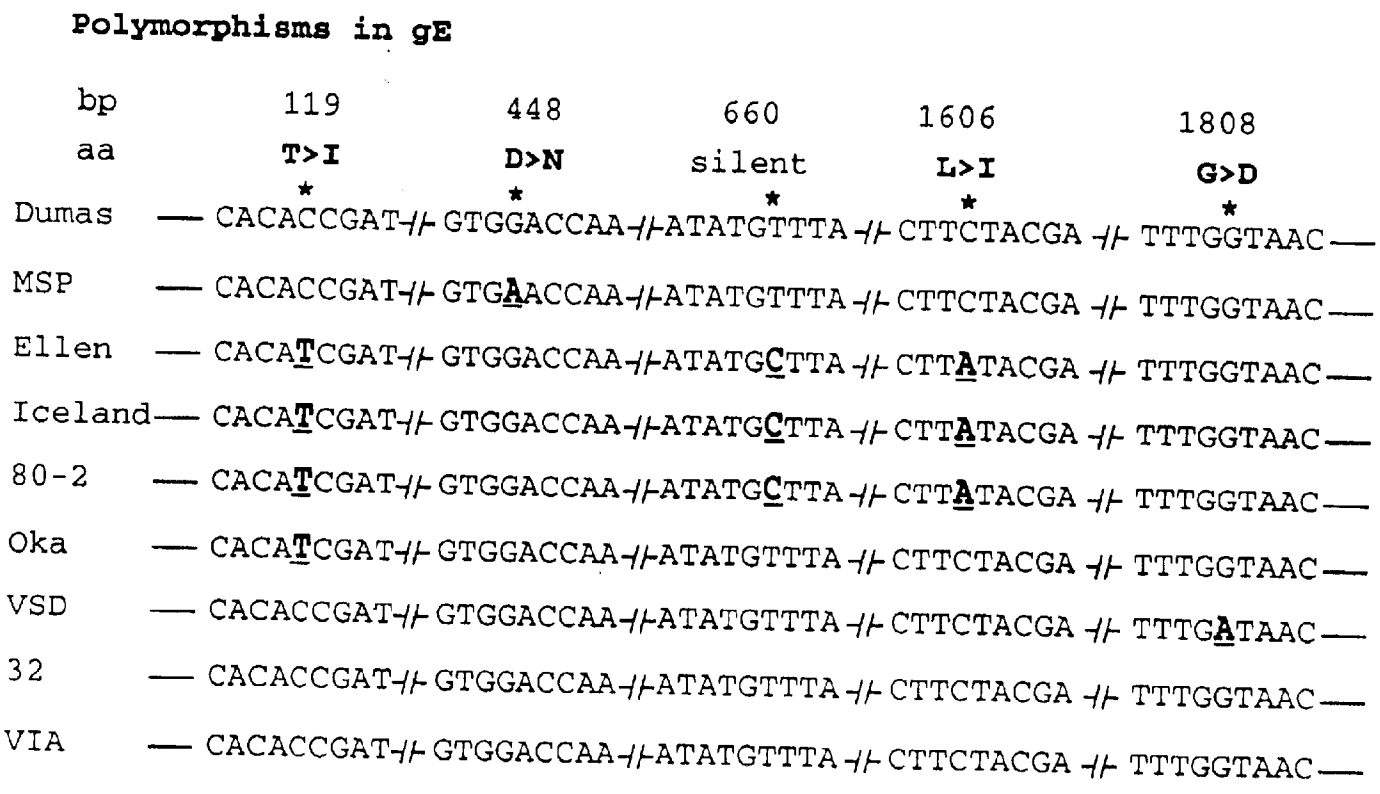

Polymorphisms in gE
bp 119 448 660 1606 1808
aa T>I D>N silent L>I G>D
Dumas — CACACCGAT-//-GTGGACCAA-//-ATATGTTTA-//-CTTCTACGA-//-TTTGGTAAC —
MSP — CACACCGAT-//-GTGAACCAA-//-ATATGTTTA-//-CTTCTACGA-//-TTTGGTAAC —
Ellen — CACATCGAT-//-GTGGACCAA-//-ATATGCTTA-//-CTTATACGA-//-TTTGGTAAC —
Iceland — CACATCGAT-//-GTGGACCAA-//-ATATGCTTA-//-CTTATACGA-//-TTTGGTAAC —
80-2 — CACATCGAT-//-GTGGACCAA-//-ATATGCTTA-//-CTTATACGA-//-TTTGGTAAC —
Oka — CACATCGAT-//-GTGGACCAA-//-ATATGTTTA-//-CTTCTACGA-//-TTTGGTAAC —
VSD — CACACCGAT-//-GTGGACCAA-//-ATATGTTTA-//-CTTCTACGA-//-TTTGATAAC —
32 — CACACCGAT-//-GTGGACCAA-//-ATATGTTTA-//-CTTCTACGA-//-TTTGGTAAC —
VIA — CACACCGAT-//-GTGGACCAA-//-ATATGTTTA-//-CTTCTACGA-//-TTTGGTAAC —

Fig. 7

Polymorphisms in gl

bp	15	546
aa	5 **Q>H**	silent
Dumas	—ATCCAATGT -//-	TCTCCGTCT—
MSP	—ATCCAATGT -//-	TCTCCGTCT—
Ellen	—ATCCAATGT -//-	TCTCCGTCT—
Iceland	—ATCCAATGT -//-	TCTCCGTCT—
80-2	—ATCCAATGT -//-	TCTCCGTCT—
Oka	—ATCCAATGT -//-	TCTCC**A**TCT—
VSD	—ATCCAATGT -//-	TCTCCGTCT—
32	—ATCCA**C**TGT -//-	TCTCCGTCT—
VIA	—ATCCA**C**TGT -//-	TCTCCGTCT—

Fig. 8

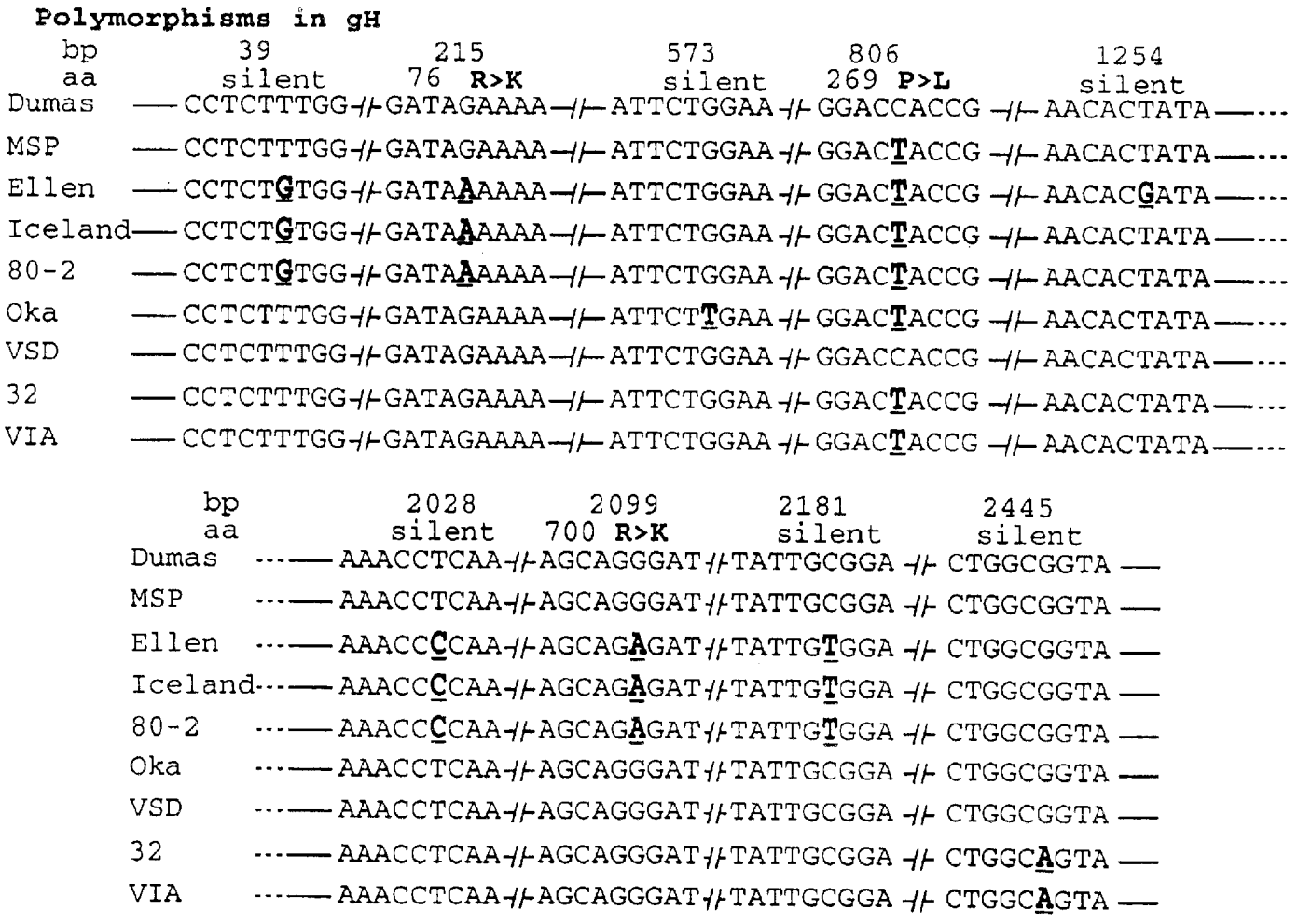

Polymorphisms in gH
bp 39 215 573 806 1254
aa silent 76 R>K silent 269 P>L silent
Dumas —— CCTCTTTGG-//-GATAGAAAA-//-ATTCTGGAA-//-GGACCACCG-//-AACACTATA——...
MSP —— CCTCTTTGG-//-GATAGAAAA-//-ATTCTGGAA-//-GGACTACCG-//-AACACTATA——...
Ellen —— CCTCTGTGG-//-GATAAAAAA-//-ATTCTGGAA-//-GGACTACCG-//-AACACGATA——...
Iceland—— CCTCTGTGG-//-GATAAAAAA-//-ATTCTGGAA-//-GGACTACCG-//-AACACTATA——...
80-2 —— CCTCTGTGG-//-GATAAAAAA-//-ATTCTGGAA-//-GGACTACCG-//-AACACTATA——...
Oka —— CCTCTTTGG-//-GATAGAAAA-//-ATTCTTGAA-//-GGACTACCG-//-AACACTATA——...
VSD —— CCTCTTTGG-//-GATAGAAAA-//-ATTCTGGAA-//-GGACCACCG-//-AACACTATA——...
32 —— CCTCTTTGG-//-GATAGAAAA-//-ATTCTGGAA-//-GGACTACCG-//-AACACTATA——...
VIA —— CCTCTTTGG-//-GATAGAAAA-//-ATTCTGGAA-//-GGACTACCG-//-AACACTATA——...
bp 2028 2099 2181 2445
aa silent 700 R>K silent silent
Dumas ...—— AAACCTCAA-//-AGCAGGGAT-//-TATTGCGGA-//-CTGGCGGTA——
MSP ...—— AAACCTCAA-//-AGCAGGGAT-//-TATTGCGGA-//-CTGGCGGTA——
Ellen ...—— AAACCCCAA-//-AGCAGAGAT-//-TATTGTGGA-//-CTGGCGGTA——
Iceland...—— AAACCCCAA-//-AGCAGAGAT-//-TATTGTGGA-//-CTGGCGGTA——
80-2 ...—— AAACCCCAA-//-AGCAGAGAT-//-TATTGTGGA-//-CTGGCGGTA——
Oka ...—— AAACCTCAA-//-AGCAGGGAT-//-TATTGCGGA-//-CTGGCGGTA——
VSD ...—— AAACCTCAA-//-AGCAGGGAT-//-TATTGCGGA-//-CTGGCGGTA——
32 ...—— AAACCTCAA-//-AGCAGGGAT-//-TATTGCGGA-//-CTGGCAGTA——
VIA ...—— AAACCTCAA-//-AGCAGGGAT-//-TATTGCGGA-//-CTGGCAGTA——

Fig. 9

Polymorphisms in gL

Dumas

	aa	8	9	10	11		106	107	108
(SEQ ID NO:77)	–	**CTG**	**CAG**	**ATA**	**GTT**	—‖—	**GTT**	**GGT**	**GAA** —
(SEQ ID NO:78)		L	Q	I	V		V	G	E

Oka

	aa	8	9	10		106	107	108
(SEQ ID NO:79)	–	**CTG**	**CAG**	**ATG**	**ATA**	—‖—	**GTT**	**GAT**	**GAA** —
(SEQ ID NO:80)		L	Q	M	I		V	D	E

Fig. 10

Polymorphisms in IE62

AA	30 *	42 S>A	61 *	129 *	131 *	172 *	190 *	195 N>D	341 *	473 *	516 *	602 A>V	609 *	628 S>G	657 A>T	688 *	703 V>A	743 *	879 *	958 R>G
Dumas	GCG	TCG	CAC	CTT	ACA	GTT	CAA	AAT	CCA	GCA	GTG	GCG	CGA	AGC	GCT	CCA	GTC	CTT	GGA	AGG
MSP	GCC	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Ellen	GCC	---	CAT	CTC	ACG	GTC	CAG	GAT	CCG	---	---	---	---	---	---	---	---	---	---	---
Iceland	GCC	GCG	CAT	CTC	---	---	---	---	---	---	---	---	---	---	---	---	GCC	---	---	GGG
80-2	GCC	---	CAT	CTC	---	---	---	---	---	---	---	---	---	---	---	---	GCC	---	---	---
OKA	GCC	---	CAT	CTC	---	---	---	---	CCG	GCG	---	---	CGG	GGC	ACT	---	GCC	---	---	---
VSD	GCC	---	---	---	---	---	---	---	---	---	---	GTG	---	---	ACT	---	---	---	---	GGG
32	GCC	---	---	---	---	---	---	---	---	---	GTA	---	---	---	---	---	---	---	---	---
VIA	GCC	---	---	---	---	---	---	---	---	---	GTA	---	---	---	---	CCG	---	CTC	GGG	GGG

AA	963 *	1035 *	1057 Q>R	1071 *	1072 Q>R	1080 *	1093 *	1143 *	1145 *	1201 H>R	1208 S>A	1208 *	1215 Q>R	1228 L>P	1241 S>G	1243 E>G	1255 S>A	1260 I>V
Dumas	TTG	GCA	CAG	CGA	CAG	GCA	GCA	GCA	CCA	CAC	TCA	TCA	CAG	CTG	AGC	GAG	TCC	ATC
MSP	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Ellen	CTG	---	---	AGA	---	---	GCG	---	---	---	---	---	---	---	---	---	---	---
Iceland	---	---	---	AGA	---	---	---	---	---	---	---	---	---	---	---	GGG	GCC	---
80-2	---	---	---	AGA	---	---	---	---	---	---	---	---	---	---	---	---	---	---
OKA	---	---	---	---	---	---	---	GCG	---	---	GCA	---	---	CCG	---	---	---	---
VSD	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
32	---	GCG	CGG	---	CGG	GCG	---	---	---	CGC	---	TCG	---	---	GGC	---	---	---
VIA	---	---	CGG	---	CGG	GCG	---	---	CCG	---	---	---	CGG	---	GGC	---	---	GTC

VARIANT VARICELLA-ZOSTER VIRUSES AND METHODS OF USE

CONTINUING APPLICATION DATA

This application is a Divisional Application of U.S. patent application Ser. No. 09/661,596, filed Sep. 14, 2000, now U.S. Pat. No. 6,528,066, which claims the benefit of U.S. Provisional Application Ser. No. 60/153,779, filed Sep. 14, 1999, all of which are incorporated by reference herein.

GOVERNMENT FUNDING

The present invention was made with government support under Grant No. AI 22795, awarded by the National Institutes of Health. The Government has certain rights in this invention.

BACKGROUND

Varicella-zoster virus (VZV) is an ancient virus. Estimations of its origins have established that the modem herpesviruses arose some 60–80 million years ago. VZV is a member of the alphaherpesvirus subfamily of herpesviridae. It is the etiologic agent of chickenpox in childhood, after which the virus enters a latent state in the dorsal root ganglia; decades later, the same virus reactivates and causes the disease shingles (herpes zoster). The entire sequence of the 125 kbp VZV genome has been published (see Davison et al., *J. Gen. Virol.*, 67:1759–1816 (1986)). With the subsequent publication of sequence data from other herpesviruses, the alphaherpesviruses have now been subdivided into two genera called Simplexvirus and Varicellovirus. VZV is considered to have one of the most stable genomes of all herpesviruses. The Oka strain of varicella vaccine derived from a Japanese child with chickenpox has a few minor genomic differences from North American strains, but to date no antigenic variation has been discovered amongst the major surface immunogens of the virion (Arvin et al., *Annu. Rev. Microbiol.*, 50:59–100 (1996)).

Based on their extensive analyses of herpesviral molecular evolutionary history, it has been estimated that herpesvirus DNA sequences mutate 10–100 times faster than the equivalent classes of sequences on the host genome. For glycoprotein gB, a highly conserved open reading frame (ORF) among all herpesviruses, it has been calculated that nonsynonymous substitutions have occurred at a rate of 2.7×10^{-8} substitutions per site per year and synonymous substitutions at 10^{-7} substitutions per site per year. Convincing arguments have been made in favor of the concept of cospeciation; in other words, herpesvirus lineages arise by way of co-evolution with their specific host. In the case of VZV, the progenitor virus most likely arose 60–70 million years before the present.

Of all the human herpesviruses, VZV may undergo the fewest replication cycles during the lifetime of the infected host. Based on a probable schema of pathogenesis, the virus actively replicates for a period of 10–14 days after infection of the human host. During a bout of chickenpox, therefore, VZV has at most 20 replication cycles. Based on the current understanding of VZV latency and reactivation, no further replication occurs unless the individual develops herpes zoster in late adulthood. Because of the above scenario, the genetic stability of the VZV genome has been presumed.

VZV contains the smallest genome of the human herpesviruses, containing about 70 ORFs within the complete VZV-Dumas sequence. Of these ORFs, at least seven code for glycoproteins, of which glycoprotein B (gB), glycoprotein E (gE), glycoprotein H (gH), and glycoprotein I (gI) are present on the exterior of the virion. VZV gE, in complex with glycoprotein I (gI), acts as a human Fc receptor on the surface of infected cells (Litwin et al., *J. Virol.*, 66:3643–51 (1992), Litwin et al., *Virology,* 178:263–72 (1990)). The cytoplasmic tails of both gE and gI contain endocytosis motifs, allowing internalization and recycling of the complex to and from the cell (Olson et al., *J. Virol.*, 71:110–119 (1997), Olson et al., *J. Virol.*, 71:4042–4054 (1992)). The gE and gI cytoplasmic tails also are modified by both serine/threonine and tyrosine phosphorylation motifs. The fact that gE cannot be deleted suggests that it is essential (Cohen et al., *Proc. Natl. Acad. Sci. USA*, 90:7376–7380 (1993), Mallory et al., *J. Virol.*, 71:8279–88 (1997)).

In VZV infection in humans, VZV gE is the most abundantly produced viral glycoprotein during infection. VZV gE is a major antigenic determinant to which numerous humoral and cytolytic responses are observed (Arvin et al., *J. Immunol.*, 137:1346–1351 (198); Bergen et al., *Viral Immunol.*, 4:151–166 (1991); and Ito et al., *J. Virol.*, 54:98–103 (1985)). Recently, an immunodominant B-cell epitope was demarcated in the gE ectodomain; the epitope is defined by murine monoclonal antibody (MAb) 3B3 (Duus et al., *J. Virol.*, 70:8961–8971 (1996); Hatfield et al., *BioTechniques* 22:332–337 (1997); and Grose, U.S. Pat. No. 5,710,248).

It has long been believed that varicella zoster virus exists in nature as a single serotype (Rentier, *Neurol.*, 45(Suppl. 8), S8 (1995), and that all varicella zoster viruses had essentially the same immunological properties. The first strain of varicella zoster virus that was sequenced was VZV-Dumas. Following the publication of this sequence, it was further believed that all varicella zoster viruses had essentially the same genetic properties as VZV-Dumas.

Significant progress has been made in the diagnosis of and vaccination against the sole VZV serotype that is believed to exist and cause disease in the United States. However, the production of the reagents used in diagnosis and vaccination of VZV is time consuming and expensive due to the slow growth rate of the strain grown to produce antigens for diagnostic and vaccine use.

SUMMARY OF THE INVENTION

The present invention represents a significant advance in the art of detecting and preventing varicella zoster virus infection and disease. During the characterization of a varicella zoster virus isolated from a patient, the surprising and unexpected observation was made that the virus had a different serotype. This strain was designated VZV-MSP. The molecular basis of the different serotype was found to be a single nucleotide polymorphism in the genome between VZV-Dumas and VZV-MSP. It was also determined that this single nucleotide polymorphism resulted in the loss of an epitope that is the epitope to which most protective antibody is produced upon vaccination with most currently used vaccines.

Typically, varicella zoster virus isolates can be divided into two groups with respect to growth rate in tissue culture cells. Some isolates, for instance VZV-Oka and VZV-Ellen, grow at a rate that results in complete lysis of a monolayer in about 5 to 7 days. Clinical isolates typically grow at a rate that results in complete lysis of a monolayer in about 4 to 5 days. Further investigation revealed that the new strain, VZV-MSP, unexpectedly and surprisingly had by in vitro tissue culture a growth rate that was significantly higher than

previously characterized isolates, and was able to lyse a monolayer in about 2 days.

The present invention provides a method for detecting antibodies that specifically bind to a varicella zoster polypeptide. A biological sample that includes an antibody is contacted with a preparation that includes a varicella zoster polypeptide, for instance an isolated varicella zoster polypeptide or fragment thereof, to form a mixture. The varicella zoster polypeptide includes a polymorphism and can encoded by a polymorphism of ORF37. The polymorphism in the polypeptide encoded by the polymorphic ORF37 can be due to a single amino acid polymorphism, which can be present in the polypeptide as a leucine at amino acid 269. Alternatively, the varicella zoster polypeptide includes a polymorphism and can encoded by a polymorphism of ORF68. The polymorphism in the polypeptide encoded by the polymorphic ORF68 can be due to a single amino acid polymorphism, which can be present in the polypeptide as an asparagine at amino acid 150. The mixture is incubated under conditions to allow the antibody to specifically bind the polypeptide to form a polypeptide:antibody complex. The presence or absence of the polypeptide:antibody complex is then detected. Detecting the polypeptide:antibody complex indicates the presence of antibodies that specifically bind to a varicella zoster polypeptide.

The preparation can include whole varicella zoster virus, for instance VZV-MSP or a modified varicella zoster virus, where the modified virus has the ATCC designation VR-795 wherein the nucleotide sequence of the virus has been modified to comprise the polymorphism of ORF37 or ORF68. The biological sample can be blood, vesicle fluid, bone marrow, brain tissue, or combinations thereof. Also provided are kits for detecting antibodies that specifically bind to a varicella zoster polypeptide. This kits include a whole varicella zoster virus.

In another aspect, the present invention provides a method for detecting the presence of a varicella zoster virus in an animal. The method includes detecting the presence of an antibody to a varicella zoster virus polypeptide encoded by a polymorphic ORF of GenBank X04370. The ORF can be ORF37 or ORF68, where the encoded polypeptide includes a single amino acid polymorphism. When the polypeptide is encoded by ORF37, the single amino acid polymorphism present in the polypeptide can be a leucine at amino acid 269. When the polypeptide is encoded by ORF68, the single amino acid polymorphism present in the polypeptide can be an asparagine at amino acid 150. Optionally, the antibody that is detected does not specifically bind to the varicella zoster polypeptide encoded by ORF37 of GenBank Accession X04370 or ORF68 of GenBank Accession X04370.

The present invention is also directed to a method for diagnosing a disease, for instance chicken pox and shingles, caused by varicella zoster virus. The method includes contacting a polynucleotide, optionally an isolated polynucleotide, of a subject suspected of having a disease caused by varicella zoster virus with a primer pair. This is incubated under conditions suitable to form a detectable amplification product, and the primer pair will not form a detectable amplification product when incubated with a polynucleotide having the nucleotide sequence of GenBank Accession X04370. An amplification product is detected, where the detection indicates that the subject has a disease caused by varicella zoster virus. The polynucleotide of the subject can be present in a biological sample, including blood, vesicle fluid, bone marrow, brain tissue, or combinations thereof.

The polynucleotide that is amplified to result in a detectable amplification product can include a single nucleotide polymorphism relative to the nucleotide sequence of GenBank Accession X04370 (SEQ ID NO:76). The primer pair can include a first primer that includes nucleotides that hybridize with a polynucleotide of GenBank Accession X04370, and a second primer comprising nucleotides that hybridize with a polynucleotide of GenBank Accession X04370, with the proviso that the 3' nucleotide of the second primer hybridizes to the single nucleotide polymorphism relative to the nucleotide sequence of GenBank Accession X04370 and does not hybridize with the corresponding nucleotide present in the nucleotide sequence of GenBank Accession X04370. The single nucleotide polymorphism can be present in ORF37, and the single nucleotide polymorphism can be present at nucleotide 806 of ORF37. The nucleotide at nucleotide 806 can be a thymine. The single nucleotide polymorphism can be present in ORF68, and the single nucleotide polymorphism can be present at nucleotide 448 of ORF68. The nucleotide at nucleotide 448 can be an adenine. An example of a primer pair is CGATGACAGACATAAAATTGTAAATGTGA (SEQ ID NO: 1) and CACCCAAGTATTGTTITTCTGTCCG (SEQ ID NO:2).

The present invention further provides a method for detecting a varicella zoster virus, for instance VZV-MSP, having a single nucleotide polymorphism in ORF68. The method includes contacting a polynucleotide with a primer pair and incubating under conditions suitable to form a detectable amplification product. The primer pair amplifies a portion of ORF68 of GenBank Accession X04370 and/or a polymorphism thereof, that includes nucleotide 448 of RF68. The amplification product is exposed to a restriction endonuclease having nucleotide 448 in its recognition sequence. Examples of restriction endonuclease include AfII, AsuI, AvaII, Cfr13I, Eco47I, NspIV, PshAI, Sau96I, and SinI. The amplification product is then detected. The presence of an amplification product that is not cleaved by the restriction endonuclease indicates the presence of a varicella zoster virus having a single nucleotide polymorphism in ORF68. The polynucleotide can be present in a biological sample, including, for instance, blood, vesicle fluid, bone marrow, brain tissue, or combinations thereof. Optionally, the polynucleotid can be isolated. An example of a primer pair is GGCATACTACCAATGACACG (SEQ D NO:12) and AAGCTCCAAGTCTCGGTGTACC (SEQ ID NO:71).

The present invention is directed to a vaccine composition that includes a modified attenuated varicella zoster virus. The modified attenuated virus has the ATCC designation VR-795, and the nucleotide sequence of the virus has been modified to contain a single nucleotide polymorphism. The single nucleotide polymorphism can be present in the coding sequence encoding glycoprotein H. For instance, the single nucleotide polymorphism in the virus can be present at nucleotide 806 of the coding sequence encoding glycoprotein H. The nucleotide present at nucleotide 806 can be a thymine. The single nucleotide polymorphism can be present in the coding sequence encoding glycoprotein E. For instance, the single nucleotide polymorphism in the virus is present at nucleotide 448 of the coding sequence encoding glycoprotein E. The nucleotide present at nucleotide 448 can be an adenine.

Also provided by the present invention is a method for producing a modified attenuated varicella zoster virus. The method includes growing the virus in a tissue culture preparation. The virus has the ATCC designation VR-795, and the nucleotide sequence of the virus has been modified to

contain a single nucleotide polymorphism. The single nucleotide polymorphism can be present in the coding sequence encoding glycoprotein H. For instance, the single nucleotide polymorphism in the virus can be present at nucleotide 806 of the coding sequence encoding glycoprotein H. The nucleotide present at nucleotide 806 can be a thymine. The single nucleotide polymorphism can be present in the coding sequence encoding glycoprotein E. The single nucleotide polymorphism in the virus can be present at nucleotide 448 of the coding sequence encoding glycoprotein E. The nucleotide present at nucleotide 448 can be an adenine. The modified attenuated virus can have an in vitro growth rate that is greater than the in vitro growth rate of a second varicella zoster virus. The second varicella zoster virus can be, for instance, VZV-32, ATCC VR-586, ATCC VR-1367, or ATCC VR-795. The growth rate of the modified varicella virus can be at least about 4-fold greater than the second varicella zoster virus at 48 hours postinfection. Optionally, the modified varicella virus can be isolated.

The present invention further provides isolated polynucleotides, including an isolated polynucleotide having the nucleotide sequence of nucleotides 66,074 to 68,599 of GenBank Accession X04370, with the proviso that nucleotide 66,879 is a thymine; and an isolated polynucleotide having the nucleotide sequence of nucleotides 115,808 to 117,679 of GenBank Accession X04370, with the proviso that nucleotide 116,255 is an adenine. Also provided are the isolated polypeptides encoded by each of the above two polynucleotides. The polynucleotide can be isolated from a varicella zoster virus.

Also provided are viruses having the designation VZV-MSP, VZV-VSD, VZV-VIA, or VZV-Iceland.

Definitions

As used herein, an antibody that can "specifically bind" a polypeptide is an antibody that interacts only with the epitope of the antigen that induced the synthesis of the antibody, or interacts with a structurally related epitope. "Epitope" refers to the site on an antigen to which specific B cells and/or T cells respond so that antibody is produced. As used herein, the term "polypeptide:antibody complex" refers to the complex that results when an antibody specifically binds to a polypeptide.

"Polypeptide" as used herein refers to a polymer of amino acids and does not refer to a specific length of a polymer of amino acids. Thus, for example, the terms peptide, oligopeptide, protein, and enzyme are included within the definition of polypeptide. This term also includes post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. Coding sequence, coding region, and open reading frame are used interchangeably and refer to a polynucleotide that encodes a polypeptide, usually via mRNA, when placed under the control of appropriate regulatory sequences. The boundaries of the coding region are generally determined by a translation start codon at its 5' end and a translation stop codon at its 3' end.

An "ORF" followed immediately by a number, for instance ORF37 or ORF68, refers to a specific open reading frame of varicella zoster virus. The approximately 70 individual open reading frames of varicella zoster virus are known to the art, and are described in Davison et al. (*J. Gen. Virol.*, 7:1759–1816 (1986)) and at GenBank Accession X04370. GenBank Accession X04370 is also referred to herein as SEQ ID NO:76. For instance, ORF37 is the open reading frame encoded by nucleotides 66,074 to 68,599 of the nucleotide sequence at GenBank Accession X04370, and ORF68 is the open reading frame encoded by nucleotides 115,808 to 117,679 of the nucleotide sequence at GenBank Accession X04370. A "polymorphic ORF" followed immediately by a number, for instance polymorphic ORF37 or polymorphic ORF68, refers to an open reading frame of varicella zoster virus that has a nucleotide sequence similar to the appropriate nucleotide sequence of GenBank X04370, but includes a single nucleotide polymorphism. Moreover, a polymorphic ORF may contain an insertion or deletion of nucleotides, preferably an insertion of 3 nucleotides or a deletion of 3 nucleotides. When referring to a specific nucleotide of an ORF, the first nucleotide of the start codon is considered to be nucleotide 1, with the following amino acids labeled consecutively. When referring herein to a specific amino acid of a polypeptide encoded by an ORF, the first methionine (prior to any post-translational modification that may occur) is considered to be amino acid 1, with the following amino acids labeled consecutively.

As used herein, the term "polynucleotide" refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxynucleotides, and includes both double- and single-stranded DNA and RNA. A polynucleotide may include nucleotide sequences having different functions, including for instance coding sequences, and non-coding sequences. A polynucleotide can be obtained directly from a natural source, for instance from a virus, or can be prepared with the aid of recombinant, enzymatic, or chemical techniques. A polynucleotide can be linear or circular in topology. A polynucleotide can be, for example, a portion of a vector, such as an expression or cloning vector, or a fragment.

An "isolated" polypeptide or polynucleotide means a polypeptide or polynucleotide that has been either removed from its natural environment, produced using recombinant techniques, or chemically or enzymatically synthesized. Preferably, a polypeptide or polynucleotide of this invention is purified, i.e., essentially free from any other polypeptide or polynucleotide and associated cellular products or other impurities. An "isolated" varicella zoster virus means a varicella zoster virus has been removed from its natural environment, e.g, the cell that produced the virus.

As used herein, the term "whole varicella zoster virus" refers to a varicella zoster virus particle or virion. The particle can be infective, i.e., be able to reproduce when introduced to an appropriate tissue culture cell under the appropriate conditions, or the particle can be inactive, i.e., incapable of reproducing.

As used herein, a "biological sample" refers to a sample of tissue or fluid isolated from a subject, including but not limited to, for example, blood, plasma, serum, lymph tissue and lymph fluid, cerebrospinal fluid, bone marrow, brain tissue, samples of the skin, external secretions of the skin including vesicle fluid from a pox, organs, biopsies and also samples of in vitro cell culture constituents including but not limited to conditioned media resulting from the growth of cells and tissues in culture medium, and cell components, or combinations thereof. A "subject" is an animal, including, for instance, a mouse or a human, preferably a human.

As used herein, the term "whole varicella zoster virus particle" refers to an intact varicella zoster virus, for instance a varicella zoster virus that has been produced by a cell and not manipulated to cause the polypeptides that make up the envelop to disassociate from one another.

As used herein, a "primer pair" refers to two single stranded polynucleotides that can be used together to amplify a region of a polynucleotide, preferably by a polymerase chain reaction (PCR). The polynucleotide that results from amplifying a region of a polynucleotide is

referred to as an "amplification product." The phrase "under conditions suitable to form a detectable amplification product" refers to the reactions conditions that result in an amplification product. For instance, in the case of a PCR, the conditions suitable to form a detectable amplification product include the appropriate temperatures, ions, and enzyme.

As used herein, the term "hybridize" refers to the ability of two complementary single stranded polynucleotides to base pair with each other, where an adenine of one polynucleotide will base pair to a thymine of a second polynucleotide and a cytosine of one polynucleotide will base pair to a guanine of a second polynucleotide. When the term "hybridize" is used to describe the interaction between a primer and a polynucleotide, hybridization requires that the 3' nucleotide of a primer be able to base pair with the corresponding nucleotide of the polynucleotide that is to be amplified. Typically, the inability of the 3' nucleotide of a primer to base pair with the polynucleotide that is to be amplified results in no amplification (see Newton et al., U.S. Pat. No. 5,595,890).

As used herein, the term "in vitro growth rate" refers to the rate at which a varicella zoster virus spreads from an infected tissue culture cell to an adjacent uninfected tissue culture cell. A tissue culture cell is a cell that replicate in vitro in a nutritive media. The in vitro growth rate of a varicella zoster virus can be measured as described herein.

As used herein, the term "vaccine composition" refers to a pharmaceutical composition containing an antigen, where the composition can be used to prevent or treat a disease or condition in a subject. "Vaccine composition" thus encompasses both subunit vaccines, as described below, as well as compositions containing whole killed, attenuated or inactivated virus. "Subunit vaccine composition" refers to a composition containing at least one immunogenic polypeptide, but not all antigens, derived from a varicella zoster virus. Such a subunit vaccine composition is substantially free of intact virus particles. Thus, a "subunit vaccine composition" is prepared from an isolated, preferably purified, immunogenic polypeptide from the virus. A subunit vaccine composition can comprise the subunit antigen or antigens of interest isolated from other antigens or polypeptides from the pathogen.

As used herein, an "attenuated varicella zoster virus" refers to a varicella zoster virus that is less virulent in humans and preferably, when introduced to a human in the appropriate manner, causes a protective immunological response such that resistance to infection will be enhanced and/or the clinical severity of the disease reduced.

As used herein, a "single nucleotide polymorphism" and a "single amino acid polymorphism" refers to a specific type of polymorphism in a polynucleotide and a polypeptide, respectively, and are described in greater detail herein.

As used herein, the term "recognition sequence" refers to the site on a polynucleotide to which a restriction endonuclease binds prior to cleaving the polynucleotide.

Unless otherwise specified, "a," "an," "the," and "at least one" are used interchangeably and mean one or more than one.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1**. Sequence of the ectodomain of VZV gE. (A) The deduced amino acid sequence of the N-terminal 400 of 623 codons of wild-type VZV gE. The previously defined MAb 3B3 epitope is underlined. The aspartic acid residue altered in VZV-MSP is designated by an arrowhead at codon 150. The silent mutation is indicated by an arrowhead at codon 341. (B) Nucleotides 149–161 and deduced amino acid sequence of the MAb 3B3 epitope in the wild type VZV-32 strain (designated VZVgE) and the mutant VZV-MSP strain gE gene (designated VZVgE-D150N). The altered nucleotides (G to A) and amino acids (D to N) are underlined and marked with the arrow. The two additional codons inserted into the expression plasmid described in FIG. **4** are designated 3B3.2, while the original 3B3 epitope is designated 3B3.11.

FIG. **2**. Recombination PCR mutagenesis. Two additional codons (3B3.2) were inserted into the MAb 3B3 epitope to produce plasmid gL 3B3.13 from plasmid gL 3B3.11.

FIG. **3**. Quantitative analysis of VZV IE 62 by confocal microscopy. VZV-MSP or VZV-32 infected monolayers were examined by confocal microscopy at increasing times post-infection at ×4 magnification. The total number of pixels positive for VZV IE 62 within each image was quantitated with the Brainvox tal_support programs (University of Iowa) as described herein. The graph summarizes the results from four separate images. Error bars: +/−1 S.D.

FIG. **4**. Infectious center assays of VZV-MSP and VZV-32.

FIG. **5**. Summary of genetic analysis of VZV-MSP (A) Schematic diagram showing regions of the VZV-MSP genome where the nucleotide, sequence has been determined. ORFs 31, 37, 47, 60, 61, 62, 66, 67, and 68 are shown. The polypeptide encoded by the ORF is shown in parentheses under the appropriate ORF. The horizontal arrows above the schematic of the VZV-MSP genome represent the location of the ORFs and the direction of transcription. U_L, unique long; IRs, internal repeat short; U_S, unique short; TRs, terminal repeat short; shaded boxes, regions of the VZV-MSP that have been sequenced; hatched boxes, repeat sequences. (B) Summary of results of sequence analysis of amplified fragments. All mutations discovered are listed by the nucleotide number of the Dumas strain. Any substitutions within open reading frames are followed by the predicted amino acid expressed by VZV-MSP. Nucleotides, nucleotides that were sequenced (the numbering system used is that described in Davison et al., (*J. Gen. Virol.*, 67:1759–1816 (1986))); Size of Region, number of nucleotides sequenced; ORF(s), the ORF or region near an ORF that was sequenced; Substitutions, locations and nature of single nucleotide polymorphism. If the single nucleotide polymorphism encodes a mutation in the resulting polypeptide, the location and nature of the mutation is shown in parentheses. For instance, at position 269 of glycoprotein gH, the proline has been replaced with a leucine.

FIG. **6**. Comparative sequence analysis of the VZV gE. VZV ORF 68 was amplified from the viral DNA of eight VZV strains, including VZV-MSP. Each sequence was compared to the prototype VZV-Dumas genotype. The location of each detected polymorphism is designated by nucleotide number (bp) of the gE gene. Any resulting single amino acid polymorphism that results in gE (e.g., T>1) is noted below the location of the appropriate detected polymorphism. Silent, the single nucleotide polymorphism did not result in a single amino acid polymorphism; asterisk, location of the single nucleotide polymorphism.

FIG. **7**. Comparative sequence analysis of VZV gI. VZV ORF 67 was amplified from viral DNA of eight VZV strains. Each sequence was compared to prototype VZV-Dumas genotype. Any resulting single amino acid polymorphism that results in gI (e.g., Q>H) is noted below the location of the appropriate detected polymorphism. Silent, the single

nucleotide polymorphism did not result in a single amino acid polymorphism.

FIG. **8**. Comparative sequence analysis of VZV gH. VZV ORF 37 was amplified from viral DNA of eight VZV strains. Each DNA sequence was compared to the prototype VZV-Dumas genotype. Nucleotide variations from the VZV-Dumas genotype were tabulated. The P269L mutation originally discovered in VZV-MSP was also present in six other VZV strains, including VZV-32. A total of nine polymorphisms within ORF 37 were discovered among the eight tested strains. Any resulting single amino acid polymorphism that results in gH (e.g., R>K) is noted below the location of the appropriate detected polymorphism. Silent, the single nucleotide polymorphism did not result in a single amino acid polymorphism.

FIG. **9**. Comparative sequence analysis of VZV gL. VZV ORF 60 was amplified from eight VZV strains. Each sequence was compared to the VZV Dumas genotype. Only the gL gene of VZV Oka differed from the gL sequence of VZV Dumas.

FIG. **10**. Comparative sequence analysis of the VZV IE 62 regulatory gene. VZV ORF 62 was amplified from eight VZV strains. Each sequence was compared to the VZV-Dumas genotype. A total of 38 polymorphisms were detected among the eight VZV strains. VZV-MSP contained only a silent substitution within codon 30 when compared to the VZV-Dumas gene. Any resulting single amino acid polymorphism that results in IE 62 (e.g., S>A) is noted below the location of the appropriate detected polymorphism. Asterisk, location of single nucleotide polymorphism that did not result in a single amino acid polymorphism

DETAILED DESCRIPTION OF THE INVENTION

Polynucleotides

The present invention provides polynucleotides, preferably isolated polynucleotides, having a (at least one) single nucleotide polymorphism. An isolated polynucleotide has a nucleotide sequence that is identical to a nucleotide sequence present in VZV-Dumas, but there is a nucleotide that is polymorphic between the isolated polynucleotide of the present invention and the corresponding polynucleotide present in VZV-Dumas. The nucleotide sequence of VZV-Dumas is present at GenBank Accession X04370. The term "polymorphism" refers to the coexistence of at least two different forms (i.e., at least two different nucleotide sequences or at least two different amino acid sequences) of a polynucleotide or a polypeptide in members of Varicella zoster. The polymorphism can be due to a single nucleotide that is different (a single nucleotide polymorphism). In contrast, a polymorphism can be due to several consecutive nucleotides, for instance 2, 3, or 4 consecutive nucleotides, that are different, which is not within the scope of the present definition of "single nucleotide polymorphism." In the isolated polynucleotides of the present invention, a polymorphism is due to, in increasing order of preference, the presence of 1 single nucleotide polymorphism, at least 1, at least 2, at least 3, most preferably at least 4 single nucleotide polymorphisms in the polynucleotide. Preferably, the isolated polynucleotides of the present invention include no greater than 4 single nucleotide polymorphisms. A single nucleotide polymorphism could be separated from another single nucleotide polymorphism by only one nucleotide.

An example of a polynucleotide of the present invention is a polymorphic ORF37 or the complement thereof, where a polymorphism is at nucleotide 806 of ORF37 (see FIG. **8**). The nucleotide at nucleotide 806 can be a guanine, adenine, or thymine, preferably a thymine.

Another example of a polynucleotide of the present invention has the nucleotide sequence of nucleotides 101, 650 to 103,081 (i.e., the region upstream of ORF60) of GenBank Accession X04370 or the complement thereof, but contains a single nucleotide polymorphism at nucleotide 102,203, or a single nucleotide polymorphism at nucleotide 102,575, or a single nucleotide polymorphism at nucleotide 102,617, or a single nucleotide polymorphism at nucleotide 102,969. The nucleotide at nucleotide 102,203 can be a guanine, cytosine, or thymine, preferably a guanine. The nucleotide at nucleotide 102,575 can be a guanine, cytosine, or thymine, preferably a guanine. The nucleotide at nucleotide 102,617 can be a guanine, adenine, or thymine, preferably a thymine. The nucleotide at nucleotide 102,969 can be a guanine, cytosine, or thymine, preferably a guanine.

A further example of a polynucleotide of the present invention has the nucleotide sequence of nucleotides 104, 468 to 104,936 (i.e., the region upstream of ORF61) of GenBank Accession X04370 or the complement thereof, but contains a single nucleotide polymorphism at nucleotide 104,898. The nucleotide at nucleotide 104,898 can be a guanine, cytosine, or thymine, preferably a guanine.

Another example of a polynucleotide of the present invention is a polymorphic ORF62 or the complement thereof, where a polymorphism is at nucleotide 90 of ORF62 (see FIG. **10**). The nucleotide at nucleotide 90 can be a guanine, adenine, or thymine, preferably a guanine.

Another example of a polynucleotide of the present invention is a polymorphic ORF66 or the complement thereof, where a polymorphism is at nucleotide 1,104 of ORF66. The nucleotide at nucleotide 1,104 can be a guanine, cytosine, or thymine, preferably a guanine.

Another example of a polynucleotide of the present invention is a polymorphic ORF68 or the complement thereof, where a polymorphism is at nucleotide 448 of ORF68 (see FIG. **6**). The nucleotide at nucleotide 448 can be an adenine, cytosine, or thymine, preferably an adenine.

Other examples of polynucleotides of the present invention are shown in FIGS. **5–10**.

The polynucleotides of the present invention can be obtained by recombinant techniques known to the art including, for instance, cloning from a member of varicella zoster or mutagenizing a polynucleotide so that it has the nucleotide sequence of a polynucleotide of the present invention. Alternatively, a polynucleotide of the present invention can be chemically or enzymatically synthesized by, for instance, an oligonucleotide synthesizer or PCR.

The present invention further includes polynucleotides that are similar to polynucleotides of the present invention as described above, including nucleotides of a polymorphic ORF37 or a polymorphic ORF68, or the complements thereof. The similarity is referred to as structural similarity and is determined by aligning the residues of the two polynucleotides (i.e., the nucleotide sequence of the candidate polynucleotide and the nucleotide sequence of a preferred polynucleotide of the invention) to optimize the number of identical nucleotides along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of shared nucleotides, although the nucleotides in each sequence must nonetheless remain in their proper order. Moreover, the nucleotide at the position of the single nucleotide polymorphism (e.g., the thymine at nucleotide 806 in a polymorphic ORF37) is invariant in the candidate polynucleotide. A candidate polynucleotide is the polynucleotide being compared to a preferred polynucleotide of the present invention. Preferably, two nucleotide sequences are com-

pared using the Blastn program, version 2.0.14, of the BLAST 2 search algorithm, as described by Tatusova, et al. (*FEMS Microbiol Lett* 1999, 174:247–250), and available at http://www.ncbi.nlm.nih.gov/gorf/b12.html. Preferably, the default values for all BLAST 2 search parameters are used, including reward for match=1, penalty for mismatch=−2, open gap penalty=5, extension gap penalty=2, gap x_dropoff=50, expect=10, wordsize=11, and filter on. In the comparison of two nucleotide sequences using the BLAST search algorithm, structural similarity is referred to as "identities." Preferably, a polynucleotide includes a nucleotide sequence having a structural similarity with a preferred polynucleotide of the present invention of at least about 98%, more preferably at least about 99%, most preferably at least about 99.5% identity.

The present invention further includes isolated polynucleotide fragments. A polynucleotide fragment is a portion of an isolated polynucleotide as described herein, where the portion is preferably at least about 15, more preferably at least about 20, most preferably at least about 25 consecutive nucleotides and includes at least one single nucleotide polymorphism. The single nucleotide polymorphism can be at any location in the polynucleotide fragment, and preferably is the nucleotide at one of the 3' ends of the fragment (when the polynucleotide fragment is double stranded) or the nucleotide at the 3' end of the fragment (when the polynucleotide fragment is single stranded).

A polynucleotide of the invention can be inserted in a vector. Construction of vectors containing a polynucleotide of the invention employs standard ligation techniques known in the art. See, for instance, Sambrook et al, *Molecular Cloning: A Laboratory Manual.*, Cold Spring Harbor Laboratory Press (1989). A vector can provide for further cloning (amplification of the polynucleotide), i.e., a cloning vector, or for expression of the polypeptide encoded by the coding sequence, i.e., an expression vector. The term vector includes, but is not limited to, plasmid vectors, viral vectors, cosmid vectors, or artificial chromosome vectors. Typically, a vector is capable of replication in a bacterial host, for instance *E. coli*. Preferably the vector is a plasmid.

Selection of a vector depends upon a variety of desired characteristics in the resulting construct, such as a selection marker, vector replication rate, and the like. Suitable host cells for cloning or expressing the vectors herein are prokaryote or eukaryotic cells. Preferably the host cell secretes minimal amounts of proteolytic enzymes. Suitable prokaryotes include eubacteria, such as gram-negative or gram-positive organisms. Preferably, *E. coli* is used.

Suitable host cells for the expression of the polypeptides of the invention, preferably encoded by a polymorphic ORF37 or a polymorphic ORF68 as described herein and containing a single amino acid polymorphism can be derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. Vertebrate or invertebrate culture can be used. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda, Aedes aegypti, Aedes albopictus, Drosophila melanogaster, Trichoplusia ni*, and *Bombyx mori* are known to the art.

Vertebrate cells can also be used as hosts. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (CAS-7, ATCC CRL-1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen. Virol.*, 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/−DHFR (CHO); CHO-K1 (ATCC CCL-61); CHO-D; mouse sertoli cells (TM4); monkey kidney cells (CV1, ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (WI 38, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL 51); TRI cells; MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Suitable plasmids for expression in *E. coli*, for example, include pUC(X), pKK223-3, pKK233-2, pTrc99A, and pET-(X) wherein (X) denotes a vector family in which numerous constructs are available. pUC(X) vectors can be obtained from Pharmacia Biotech (Piscataway, N.H.) or Sigma Chemical Co. (St. Louis, Mo.). pKK233-3, pKK233-2 and pTrc99A can be obtained from Pharmacia Biotech. pET-(X) vectors can be obtained from Promega (Madison, Wis.) Stratagene (La Jolla, Calif.) and Novagen (Madison, Wis.). To facilitate replication inside a host cell, the vector preferably includes an origin of replication (known as an "ori") or replicon. For example, Co1E1 and P15A replicons are commonly used in plasmids that are to be propagated in *E. coli.*

Suitable plasmids for expression in eukaryotic cells, for example, include the EPITAG vectors available from Invitrogen (Carlsbad, Calif.) for mammalian cells. Examples of suitable EPITAG vectors include pcDNA3.1/myc-His and pEF1/myc-His. Other plasmids that can be used in mammalian cells include, for example, pRc/RSV (Invitrogen) and pSecTag2 (Invitrogen). Suitable plasmids for expression in insect cells include, for instance, pIZ/V5-His (Invitrogen), and pBlueBac4.5 (Invitrogen).

An expression vector optionally includes regulatory sequences operably linked to the coding sequence. The invention is not limited by the use of any particular promoter, and a wide variety are known. Promoters act as regulatory signals that bind RNA polymerase in a cell to initiate transcription of a downstream (3' direction) coding sequence. The promoter used in the invention can be a constitutive or an inducible promoter. It can be, but need not be, heterologous with respect to the host cell. Preferred promoters for bacterial transformation include lac, lacUV5, tac, trc, T7, SP6 and ara.

Promoter sequences are known for eukaryotes. Most eukaryotic coding sequences have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is the CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be a signal for addition of the poly A tail to the 3' end of the coding sequence. All these sequences are suitably inserted into eukaryotic expression vectors.

Transcription of a coding sequence encoding a polypeptide of the present invention in mammalian host cells can be controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, and Hepatitis-B virus.

Transcription of a coding sequence encoding a polypeptide of the present invention by eukaryotes can be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually having about 10 to 300 bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation- and position-independent, having been found 5' and 3' to coding

sequences, within an intron as well as within the coding sequence itself. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, alpha-fetoprotein, and insulin). Enhancers from eukaryotic cell viruses are also known and include the SV40 enhancer on the late side of the replication origin, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the coding sequence encoding a polypeptide of the present invention, but is preferably located at a site 5' of the promoter.

An expression vector can optionally include a ribosome binding site (a Shine Dalgarno site for prokaryotic systems or a Kozak site for eukaryotic systems) and a start site (e.g., the codon ATG) to initiate translation of the transcribed message to produce the enzyme. It can also include a termination sequence to end translation. A termination sequence is typically a codon for which there exists no corresponding aminoacetyl-tRNA, thus ending polypeptide synthesis. The polynucleotide used to transform the host cell can optionally further include a transcription termination sequence. The rrnB terminators, which is a stretch of DNA that contains two terminators, T1 and T2, is an often used terminator that is incorporated into bacterial expression systems. Transcription termination sequences in vectors for eukaryotic cells typically include a polyadenylation signal 3' of the coding sequence.

The polynucleotide used to transform the host cell optionally includes one or more marker sequences, which typically encode a molecule that inactivates or otherwise detects or is detected by a compound in the growth medium. For example, the inclusion of a marker sequence can render the transformed cell resistant to an antibiotic, or it can confer compound-specific metabolism on the transformed cell. Examples of a marker sequence are sequences that confer resistance to kanamycin, ampicillin, chloramphenicol, tetracycline, neomycin, and formulations of phleomycin D1 including, for example, the formulation available under the trade-name ZEOCIN (Invitrogen).

Polypeptides

The present invention is also directed to polypeptides, preferably isolated polypeptides, encoded by polynucleotides of the present invention. A polypeptide has an amino acid sequence that is identical to an amino acid sequence encoded by a coding sequence present in VZV-Dumas, but there is an amino acid that is polymorphic between the polypeptide of the present invention and the corresponding polypeptide encoded by VZV-Dumas. The polymorphism can be due to a single amino acid that is different (a single amino acid polymorphism). In contrast, a polymorphism can be due to several consecutive amino acids, for instance 2, 3, or 4 consecutive amino acids, that are polymorphic, which is not within the scope of the present definition of "single amino acid polymorphism." In the polypeptides of the present invention, a polymorphism is due to, in increasing order of preferance, the presence of 1 single amino acid polymorphism, at least 1, at least 2, at least 3, most preferably at least 4 single amino acid polymorphisms in the polypeptide. Preferably, the isolated polypeptides of the present invention include no greater than 4 single amino acid polymorphisms. A single amino acid polymorphism could be separated from another single amino acid polymorphism by only one amino acid.

Preferably, a polypeptide of the present invention has immunogenic activity. "Immunogenic activity" refers to an amino acid sequence which elicits an immunological response in a subject. An immunological response to a polypeptide is the development in a subject of a cellular and/or antibody-mediated immune response to the polypeptide fragment. Usually, an immunological response includes but is not limited to one or more of the following effects: the production of antibodies, B cells, helper T cells, suppressor T cells, and/or cytotoxic T cells, directed specifically to an epitope or epitopes of the polypeptide fragment.

The polypeptides of the present invention can be obtained from, for instance, a biological sample from a subject infected with a varicella zoster virus that encodes the polypeptide. The polypeptide can be obtained from tissue culture cells that have, for instance, been infected with a varicella zoster virus that encodes the polypeptide or contain a recombinant polynucleotide, preferably a polynucleotide of the invention, that encodes the polypeptide of the invention. Alternatively, the polypeptide can be obtained from a prokaryotic cell, for instance *Eschericia coli*, that contains a recombinant polynucleotide, preferably a polynucleotide of the invention, that encodes the polypeptide of the invention. The polypeptides of the present invention can also be obtained by chemical synthesis.

An example of a polypeptide of the present invention is encoded by a polymorphic ORF37, where a polymorphism is at amino acid 269 (see FIG. **8**). When referring herein to a specific amino acid of a polypeptide encoded by an open reading frame, the first methionine is considered to be amino acid 1, with the following amino acids labeled consecutively. The polypeptide encoded by ORF37 is referred to in the art as glycoprotein H, gH, and gpIII. Preferably, the amino acid at position 269 in the polypeptide encoded by a polymorphic ORF37 is an amino acid other than proline, more preferably, the amino acid is a nonpolar (hydrophobic) amino acid, for instance alanine, leucine, isoleucine, valine, phenylalanine, tryptophan, or tyrosine, most preferably, the amino acid is leucine.

An example of a polypeptide of the present invention is encoded by a polymorphic ORF68, where a polymorphism is at amino acid 150 (see FIG. **6**). The polypeptide encoded by nucleotides ORF68 is referred to in the art as glycoprotein E, gE, and gpI. Preferably, the amino acid at position 150 in the polypeptide encoded by a polymorphic ORF68 is an amino acid other than aspartic acid, more preferably, the amino acid is asparagine, lysine, histidine, or glutamic acid, most preferably, the amino acid is asparagine.

Other examples of polypeptides of the present invention are shown in FIGS. **5–10**.

The present invention further includes polypeptides having similarity with the polypeptides of the present invention as described above, including the polypeptide encoded by a polymorphic ORF37 or a polymorphic ORF68. The similarity is referred to as structural similarity and is generally determined by aligning the residues of the two amino acid sequences (i.e., a candidate amino acid sequence and the amino acid sequence of a preferred polypeptide of the present invention) to optimize the number of identical amino acids along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of identical amino acids, although the amino acids in each sequence must nonetheless remain in their proper order. Moreover, the amino acid at the position of the single amino acid polymorphism (e.g., the leucine at amino acid 269 in the polypeptide encoded by a polymorphic ORF37) is invariant in the candidate polypeptide. A candidate amino acid sequence is the amino acid sequence being compared to an amino acid sequence present in a preferred polypeptide of the present invention.

Preferably, two amino acid sequences are compared using the Blastp program, version 2.0.14, of the BLAST 2 search algorithm, as described by Tatusova et al. (*FEMS Microbiol. Lett.*, 174:247–250 (1999)), and available at http://www.ncbi.nlm.nih.gov/gorf/bl12.html. Preferably, the default values for all BLAST 2 search parameters are used, including matrix BLOSUM62; open gap penalty=11, extension gap penalty=1, gap x_dropoff=50, expect=10, wordsize=3, and filter on. In the comparison of two amino acid sequences using the BLAST search algorithm, structural similarity is referred to as "identities." Preferably, a polypeptide includes an amino acid sequence having a structural similarity with a preferred polypeptide of the present invention of, in increasing order of preference, at least about 96%, at least about 97%, at least about 98%, and most preferably, at least about 99% identity.

The present invention further includes polypeptide fragments. A polypeptide fragment is a portion of a polypeptide as described herein, where the portion includes at least one single amino acid polymorphism. Preferably, the polypeptide fragment has immunogenic activity. Preferably, a polypeptide fragment is at least about 8, more preferably at least about 12, most preferably at least about 20 amino acids in length.

Viruses

The present invention further provides isolated varicella zoster viruses. Preferably, the genome of an isolated varicella zoster virus of the present invention includes, in increasing order of preference, 1, at least 1, at least 2, at least 3, most preferably at least 4 single nucleotide polymorphisms when compared to the nucleotide sequence of GenBank Accession X04370. Preferably, the genome of an isolated varicella zoster virus of the present invention includes no greater than 4 single nucleotide polymorphisms. Examples of isolated varicella zoster viruses of the present invention include VZV-MSP, VZV-VSD, VZV-VIA, VZV-Iceland. Alternatively, the isolated varicella zoster virus can be a modified varicella zoster virus, preferably a modified attenuated varicella zoster virus. Modified varicella zoster viruses are described in greater detail herein.

A single nucleotide polymorphism can be present in a coding sequence where it can result in the encoded polypeptide containing a single amino acid polymorphism when compared to the polypeptides encoded by the nucleotide sequence of GenBank X04370. Alternatively, a single nucleotide polymorphism can be silent, i.e., not alter the amino acid sequence of a polypeptide encoded by a coding sequence. A single nucleotide polymorphism can be present in a region of the genome that is not a coding sequence. In an isolated varicella zoster virus of the present invention that encodes a polypeptide having a single amino acid polymorphism, the varicella virus may have a serotype that is different than the serotype known to the art. Preferably, the serotype of an isolated varicella zoster virus of the invention is one that does not contain the epitope to which the monoclonal antibody 3B3 binds. Monoclonal antibody is available from the ATCC (accession number HB-12377). An example of a varicella zoster virus having this serotype is VZV-MSP.

Preferably, the isolated varicella zoster viruses of the present invention have the ability to spread from one cell to another at a rate that is greater than previously characterized varicella zoster viruses. This phenotype, which is also referred to herein as in vitro growth rate and cell-to-cell spread, can be measured by methods that are known to the art, including, for instance, the methods described in Example 2 (i.e., laser scanning confocal microscopy combined with pixel intensity measurement, infectious center assays, and replication in the SCID-hu mouse). Examples of previously characterized varicella zoster viruses that can be used as a baseline for measuring the in vitro growth rate of an isolated varicella virus of the present invention include VZV-32, Oka strain (see Kubo, U.S. Pat. No. 3,985,615), or the varicella zoster viruses having the designations ATCC VR-586, ATCC VR-1367, or ATCC VR-795. Examples of tissue culture cells that can be used include human melanoma cells (including, for instance, MeWo cells), lung fibroblasts (including, for instance, MRC-5 cells, which have the ATCC designation CCL-171), cells derived from human embryos, simian cells, or guinea pig cells.

Preferably, when the infectious center assay is used to measure the in vitro growth rate of a varicella zoster virus, tissue culture cells are added to the well of a 35-mm tissue culture plate and grown until they form a substantially confluent monolayer. The well is inoculated with between about 300 infectious centers to about 700 infectious centers, preferably about 500 infectious centers, i.e., an aliquot of the appropriate varicella zoster virus to result in the initial infection of about 500 cells. The resulting number of infectious centers in the well is measured at 24 hours after inoculation and at 48 hours after inoculation. Preferably, the number of infectious centers of a varicella zoster virus at 48 hours after inoculation is at least about 1.5-fold greater, more preferably at least about 2-fold greater, most preferably at least about 3-fold greater than a previously characterized varicella zoster virus.

Preferably, when laser scanning confocal microscopy combined with pixel intensity measurement is used to measure the in vitro growth rate of a varicella zoster virus, tissue culture cells are inoculated with the varicella zoster virus to be measured. These infected cells are then used to inoculate uninfected cells at a 1:8 ratio of infected to uninfected cells. The spread of the varicella zoster virus is then determined at 24 hours after inoculation at the 1:8 ratio and at 48 hours after inoculation at the 1:8 ratio. The spread of the varicella zoster virus away from a single cell that initially contained the virus can be measured by assaying for evidence of virus in adjacent cells. For instance, the presence of viral nucleic acid or a viral encoded polypeptide can be measured. Preferably, the presence of a viral encoded polypeptide is measured. Preferably, the viral encoded polypeptide is IE62. Preferably, the spread of a varicella zoster virus at 24 hours after inoculation at the 1:8 ratio is at least about 1.5-fold greater, more preferably at least about 2-fold greater than a previously characterized varicella zoster virus. Preferably, the spread of a varicella zoster virus at 48 hours after inoculation at the 1:8 ratio is at least about 2-fold greater, more preferably at least about 4-fold greater than a previously characterized varicella zoster virus.

The present invention is also directed at modifying a varicella zoster virus so that it has an in vitro growth rate that is greater than the in vitro growth rate prior to modification. A varicella zoster virus can be modified by altering the genome of the varicella zoster virus. Preferably, the genome is modified to contain, in increasing order of preference, 1 single nucleotide polymorphism, at least 1, at least 2, at least 3, most preferably, at least 4 single nucleotide polymorphisms. Preferably, the genome is modified to include no greater than 4 single nucleotide polymorphisms. The single nucleotide polymorphisms that could be incorporated into the genome of a varicella zoster virus are described herein. Methods of modifying a genome of a varicella zoster virus are known to the art (see, for instance, Cohen et al., *Proc. Natl. Acad. Sci. USA*, 90:7376–7380 (1993)). Preferably,

recombinant DNA techniques are used to make the modification. Preferably, the single nucleotide polymorphisms that could be incorporated into a varicella zoster virus include nucleotide 806 of ORF37, where the single nucleotide polymorphism is a thymine, and/or nucleotide 448 of ORF68, where the single nucleotide polymorphism is an adenine. Examples of varicella zoster viruses that could be modified include a clinical isolate, Oka strain (see Kubo, U.S. Pat. No. 3,985,615), ATCC VR-586, ATCC VR-1367, or ATCC VR-795, preferably ATCC VR-795. It is expected that varicella zoster virus that is presently used to produce, for instance, antigen for diagnostic assays or whole virus for use in vaccine compositions, can be modified by this method. Diagnostic assays and vaccine compositions are described in greater detail herein. The modified virus will grow at a faster rate and result in lowered production costs.

Another aspect of the present invention is directed to methods for producing a varicella zoster virus that has a high in vitro growth rate. Preferably, the varicella zoster virus has an in vitro growth rate that is greater than the in vitro growth rate of a second varicella zoster virus, including, for instance, a clinical isolate, Oka strain (see Kubo, U.S. Pat. No. 3,985,615), VZV-32, ATCC VR-586, ATCC VR-1367, or ATCC VR-795. The method can further include isolation of the varicella virus that has the high in vitro growth rate.

Methods of use

The present invention provides methods for detecting a varicella zoster virus. These methods are useful in, for instance, detecting a varicella zoster virus in an animal, diagnosing a disease caused by a varicella zoster virus, and detecting a varicella zoster virus having a single nucleotide polyporphism. Preferably, such diagnostic systems are in kit form. Kits are described in greater detail herein. In some aspects of the invention, preferably the varicella zoster virus detected is one having a serotype that is different than VZV-32, or the varicella zoster viruses having the designations ATCC VR-586, ATCC VR-1367, or ATCC VR-795, or having a single nucleotide polymorphism when compared to the nucleotide sequence of GenBank Accession X04370. Preferably, the varicella zoster virus detected is one to which the monoclonal antibody 3B3 does not bind. In some aspects of the invention, detecting a varicella zoster virus includes detecting antibodies that specifically bind to a varicella zoster polypeptide. Whether an antibody specifically binds a polypeptide or non-specifically binds a polypeptide can be determined using methods that are known in the art. Preferably, the polypeptide is gE, gH, gB, or IE62, most preferably gE. The methods include contacting an antibody with a preparation that includes a varicella zoster polypeptide to result in a mixture. Preferably, the antibody is present in a biological sample, more preferably blood, vesicle fluid, bone marrow, or brain tissue.

In this aspect of the invention the varicella zoster virus polypeptide contains a polymorphism. Such polypeptides are described herein. Preferably, the varicella zoster virus polypeptide is encoded by a polymorphic ORF68, and the encoded polypeptide includes an asparagine at amino acid 150. Alternatively and optionally, the varicella zoster virus polypeptide is encoded by a polymorphism of ORF37, and the encoded polypeptide includes a leucine at amino acid 269. The varicella zoster polypeptide in the preparation can be an isolated varicella zoster polypeptide or fragment thereof. Alternatively, preparation can further include whole varicella zoster virus, preferably VZV-MSP, VZV-VSD, VZV-VIA, or VZV-Iceland, more preferably, VZV-MSP.

The method further includes incubating the mixture under conditions to allow the antibody to specifically bind the polypeptide to form a polypeptide:antibody complex. The preparation that includes the varicella zoster virus may also includes reagents, for instance a buffer, that provide conditions appropriate for the formation of the polypeptide:antibody complex. The polypeptide:antibody complex is then detected. The detection of antibodies is known in the art and can include, for instance, immunofluorescence and peroxidase.

The methods for detecting the presence of antibodies that specifically bind to a varicella zoster polypeptide can be used in various formats that have been used to detect antibody to varicella zoster virus, including complement fixation, indirect fluorescent antibody, fluorescent antibody to membrane antigen, neutralization, indirect hemagglutination, immune adherence hemagglutination, radioimmunoassay, latex agglutination, and enzyme-linked immunosorbent assay.

Other methods for detecting a varicella zoster virus include the amplification of a polynucleotide, preferably by PCR. The polynucleotide can be one that is, for instance, isolated from a subject, preferably a subject suspected of having a disease caused by varicella zoster virus. Preferably, the polynucleotide is from a subject, for instance a biological sample, preferably blood, vesicle fluid, bone marrow, or brain tissue. In some aspects of the invention, the method includes contacting a polynucleotide, preferably an isolated polynucleotide, with a primer pair, incubating under conditions suitable to form a detectable amplification product, and detecting the amplification product. Detection indicates that the subject has a disease caused by varicella zoster virus.

The primer pair is one that will not form a detectable amplification product when incubated with a polynucleotide having the nucleotide sequence of GenBank Accession X04370, and preferably will form a detectable amplification product with a polynucleotide containing a single nucleotide polymorphism described herein. Preferably, one of the primers of the primer pair has a nucleotide sequence that hybridizes to a nucleotide sequence of GenBank Accession X04370; however, the 3' nucleotide of the primer corresponds to a single nucleotide polymorphism present in the varicella zoster virus that is to be detected. This method is known to the art as amplification refractory mutation system (ARMS; see Newton et al, U.S. Pat. No. 5,595,890). For instance, a primer pair could be CGATGACAGACATAAAATTGTAAATGTGA (SEQ ID) NO: 1), where the underlined nucleotide corresponds to the single nucleotide polymorphism present in VZV-MS in the coding sequence of nucleotides 115,808 to 117,679 (i.e., the polymorphic ORF68 coding sequence), and CACCCAAGTATTGTTTTCTGTCCG (SEQ ID NO:2). Optionly, an additional amplification can be done to detect a varicella zoster virus that does not have the single nucleotide polymorphism by using, for instance, a primer pair that will form a detectable amplification product when incubated with a polynucleotide having the nucleotide sequence of GenBank Accession X04370. An example of such a primer pair is CGATGACAGACATAAAATTGTAAATGTGG (SEQ ID NO:3), and CACCCAAGTATITGTTTTTCTCTGTCCG (SEQ ID NO:2). Other primer pairs can be designed using methods known to the art to detect other single nucleotide polymorphisms described herein.

In another aspect of the invention that involves detecting a varicella zoster virus by amplification of a polynucleotide, preferably by PCR, the method is directed to detecting a varicella zoster virus having a single nucleotide polymorphism, preferably at nucleotide 448 of ORF68. Preferably, in the varicella zoster virus to be detected,

nucleotide 448 of ORF68 is a cytosine, thymine, or adenine, more preferably an adenine. The method includes contacting a polynucleotide with a primer pair and incubating under conditions suitable to form a detectable amplification product. The amplification product is then exposed to a restriction endonuclease, preferably one that has the recognition sequence that includes nucleotide 448 and is no longer able to cleave when that nucleotide of the recognition sequence is not a guanine. Examples of such restriction endonuclease are AflI, AsuI, AvaII, Cfr13I, Eco47I, NspIV, PshAI, Sau96I, and SinI. In VZV-Dumas and other varicella zoster viruses, the nucleotide at position 448 of ORF68 in the viral genome is a guanine, and is cleaved by the above-identified restriction endonucleases. When the nucleotide at position 448 of a polymorphic ORF68 is a cytosine, thymine, or adenine, more preferably an adenine, the restriction endonuclease is no longer able to cleave the amplification product. Thus, the method further includes detecting the amplification product after exposure to the restriction endonuclease. The presence of an amplification product that is not cleaved by, for instance, AvaII, indicates the presence of a varicella zoster virus having a single nucleotide polymorphism at nucleotide 448.

The primer pair that is used in this aspect of the invention must amplify a region of varicella zoster virus genomic DNA that includes nucleotide 116,255. With out intending to be limiting, an example of a primer pair includes GGCATACTACCAATGACACG (SEQ ID NO:12) and AAGCTCCAAGTCTCGGTGTACC (SEQ ID NO:71), as well as some of the primers listed in Table 1. Other primers can be designed using methods known in the art.

The methods that involve detecting a varicella zoster virus by amplification of a polynucleotide, preferably by PCR, can also be used to determine the percentage of a population that has a particular single nucleotide polymorphism. Methods of screening populations for the presence of a single nucleotide polymorphism are known to the art. For instance, PCR is sensitive enough to allow samples from a large number of subjects to be pooled and assayed for the presence of a varicella zoster virus having a single nucleotide polymorphism.

The present invention also provides a kit for detecting a varicella zoster virus. The kit includes a varicella zoster polypeptide as described herein (when detecting antibody to varicella zoster virus) or a primer pair as described herein (when amplifying a polynucleotide) in a suitable packaging material in an amount sufficient for at least one assay. Optionally, other reagents such as buffers and solutions needed to practice the invention are also included. Instructions for use of the packaged polypeptide or primer pair are also typically included.

As used herein, the phrase "packaging material" refers to one or more physical structures used to house the contents of the kit. The packaging material is constructed by well known methods, preferably to provide a sterile, contaminant-free environment. The packaging material has a label which indicates that the polypeptide or primer pair can be used for detecting a varicella zoster virus. In addition, the packaging material contains instructions indicating how the materials within the kit are employed to detect a varicella zoster virus. As used herein, the term "package" refers to a solid matrix or material such as glass, plastic, paper, foil, and the like, capable of holding within fixed limits a polypeptide or a primer pair. Thus, for example, a package can be a glass vial used to contain milligram quantities of a primer pair, or it can be a microtiter plate well to which microgram quantities of a polypeptide have been affixed. "Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter, such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions, and the like.

The present invention is also directed to vaccines. In one aspect, the present invention is directed to vaccine compositions. Preferably, the subject receiving the vaccine composition will display a protective immunological response such that resistance to infection will be enhanced and/or the clinical severity of the disease reduced. A vaccine composition can include a modified varicella zoster virus, more preferably a modified attenuated varicella zoster virus. A varicella zoster virus can be modified as described above under "Viruses." In other alphaherpesviruses, for instance, pseudorabies virus (PRV), it has been found that spread of the virus in an infected animal is facilitated by gE mutations that reduce virulence (Yang et al., *J. Virol.*, 73:4350 (1999)), it has been found that increasing the in vitro growth rate does not result in an increased virulence of the virus. It is expected that the varicella zoster viruses used as a source of viral antigen for vaccination can be modified to have an increased in vitro growth rate, and not have a increase in virulence. Preferably, the varicella zoster virus that is modified to have an increased in vitro growth rate is Oka strain (see Kubo, U.S. Pat. No. 3,985,615), or ATCC VR-795. The modified varicella zoster virus of the vaccine composition can a live virus, or an inactivated whole virus preparation. The virulence of a varicella zoster virus modified to have a higher in vitro growth rate can be determined using methods known in the art, for instance by using human volunteers.

In another aspect, the vaccine composition can include an isolated varicella zoster virus polypeptide of the present invention or a fragment thereof. Varicella zoster virus polypeptides of the present invention are described herein.

The vaccine composition includes polypeptide or modified varicella zoster viruses having immunogenic acivity. Immunogenic carriers can be used to enhance the immunogenicity of the polypeptide or modified varicella zoster viruses. Such carriers include but are not limited to other polypeptides, polysaccharides, liposomes, and bacterial cells and membranes. Polypeptide carriers may be joined to the polypeptides or modified varicella zoster viruses of the present invention to form fusion polypeptides by recombinant or synthetic means or by chemical coupling. Useful carriers and means of coupling such carriers to polypeptide antigens are known in the art.

The vaccine compositions may be formulated by means known in the art. The formulations include those suitable for parental (including subcutaneous, intramuscular, intraperitoneal, and intravenous administration. They are typically prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The composition may also, for example, be emulsified, or the polypeptide or modified varicella zoster virus encapsulated in liposomes. Where mucosal immunity is desired and the vaccine includes a polypeptide or an inactivated varicella virus, the vaccine compositions may advantageously contain an adjuvant such as the nontoxic cholera toxin B subunit (see, e.g., U.S. Pat. No. 5,462,734). Cholera toxin B subunit is commerically available, for example, from Sigma Chemical Company, St. Louis, Mo. Other, suitable adjuvants are available and may be substituted therefor.

The polypeptide or modified varicella zoster virus can be mixed with pharmaceutically acceptable excipients or car-

riers. Suitable excipients include but are not limited to water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine compositions may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Such additional formulations and modes of administration as are known in the art may also be used.

The present invention is illustrated by the following examples. It is to be understood that the particular examples, materials, amounts, and procedures are to be interpreted broadly in accordance with the scope and spirit of the invention as set forth herein.

EXAMPLE 1

Identification of Single Nucleotide Polymorphisms in ORF 68 (the gE Gene) of VZV-MSP

This example demonstrates the presence of a single nucleotide polymorphism in gE. Because of the important functions of gE, this discovery was completely unexpected. This example provides a more complete characterization of the altered biological properties and genetic composition of this contemporary variant in VZV evolution. For the first time, a VZV variant virus has been discovered which has a cell-to-cell spread phenotype clearly distinguishable from previously characterized VZV strains.

Materials and Methods

Viruses and Cells

The mutant VZV was isolated from a 6 year old boy with leukemia, who contracted chickenpox and was hospitalized for intravenous acyclovir treatment in late 1995. The child's illness responded to treatment and no unusual sequelae were observed. The child's vesicle fluid was inoculated onto MRC-5 cells in glass tubes. The isolate was designated VZV-MSP because the child lived in Minnesota. The VZV-32 laboratory strain was isolated in Texas in 1976 from an otherwise healthy child with chickenpox (Grose, *Virology*, 101:1–9 (1980)). This virus has never been passaged more than 20 times. The VZV Oka strain was isolated from a Japanese child with chickenpox and attenuated by M. Takahashi in Japan in the 1970s (Takahashi hi et al., *Biken J.*, 18:25–33 (1975)). All viruses were subcultured in either MRC-5 cells or human melanoma cells (MeWo strain, available from C. Grose, University of Iowa, Iowa). MeWo cells are highly permissive for VZV replication but no infectious virus is released into the culture medium (Grose, *Virology*, 101:1–9 (1980)). Therefore, transfer of infectivity is carried out by trypsin dispersion of infected cells and relayering of infected cells onto an uninfect monolayer at a ratio of 1:8 (infected to uninfected cells). The HSV-1 Miyama strain was propagated in the FL line derived from human amnion cells (Padilla et al, *J. Elect. Microscopy*. 46:171–180 (1997)).

Antibodies and Immunodetection by Confocal Microscopy

MAb 3B3 was produced in this laboratory (Grose et al., *Infect. Immun.*, 40:381–388 (1983)). The antigen for mouse immunization was VZV-32 infected cells. MAb 3B3 attaches to VZV gE even under stringent conditions of buffers containing 1% SDS. Other monoclonal antibodies to VZV gE (MAb 711), VZV gI (MAb 6B5) and VZV gH (MAb 206) were also produced and characterized in this laboratory and are described in Grose (*Annu. Rev. Microbiol*, 44: 59–80 (1990). Conditions for immunodetection of VZV proteins by laser scanning confocal microscopy have been outlined by Duus et al., (*J. Virol.*, 70:8961–8971 (1996)). Immunoblotting was performed with the above antibodies as described (Grose, *Annu. Rev. Microbiol*, 44:59–80 (1990)).

Epitope Mapping by Recombination PCR Mutagenesis

The technique of recombination PCR mutagenesis has been adapted to investigate epitope mapping and tagging (Yao et al., *J. Virol.*, 67:305–314(1993), Hatfield et al., *BioTechniques*, 22:332–337 (1997)). By this methodology, the epitope of MAb 3B3 was initially defined between amino acids 151–161 in the ectodomain of the 623-amino acid gE glycoprotein. The methodology for producing plasmid pTM1-VZV gL 3B3.11 is described in detail by Hatfield et al., (*BioTechniques*, 22:332–337 (1997)). As part of the investigation in Results (FIG. **2**), an additional two codons were inserted at the N-terminus of the 11-amino acid 3B3 epitope, to produce a 13-amino acid epitope tag in the VZV gL protein. The mutating primers included the following: MP23 (sense) CAT ACT GTG TCG ACC AAA GGC AAT ACG TG ACG TG (SEQ ID NQ:4) and MP24 (antisense) TTG TG CCT TIG GTC GAC ACA GTA TGC GAT TGT GAT AG (SEQ ID NO:5). PCR amplification was performed under the following parameters: 94° C. denaturation for 30 seconds, 50° C. annealing for 30 seconds, 72° C. extension for 5 minutes; after 25 cycles, there was a final extension at 72° C. for 7 minutes. PCR products ere combined and transformed into cells where the overlapping regions underwent recombination to yield a plasmid containing the mutagenized insert. Plasmid purification was performed with a Qiagen Maxi Kit. The newly designated pTM1-VZV gL 3B3.13 plasmid was partially sequenced at the University of Iowa DNA Core Facility to confirm the authenticity of the insertional mutagenesis. This PCR mutagenesis protocol is very reliable with a error frequency of less than 0.25% (Jones et al., *Biotechniques*, 8:178–183 (1990)).

Subcloning of VZV-MSP gE

The subcloning of wild type VZV gE has been described previously (Yao et al., *J. Virol.*, 67:305–314 (1993)). Briefly, two flanking PCR primers were utilized which amplified the VZV-MSP gE ORF directly from VZV-MSP infected melanoma cells. These primers also created a Sac I and a Spe I restriction enzyme site at the 5' and 3' ends, respectively. The primers were the following: Nco gpI (sense) CGA CCC GGG GAG CTC CCA TGG GGA CAG TTA ATA AAC C (SEQ ID NO:6) and IP2 (anti-sense) CGC TCT AGA ACT AGT GGA TCC CCC GGG GAA TTT GTC ACA GGC TTT T (SEQ ID NO:7). PCR amplification was performed using AmpliTaq (Applied Biosystems, Foster City, Calif.) under the following conditions: 94° C. denaturation for 40 seconds, 50° C. annealing for 40 seconds, 72° C. extension for 4 minutes; after 35 cycles, there was a final extension at 72° C. for 7 minutes. After amplification, the PCR fragment was digested with Sac I and Spe I before cloning into the multiple cloning site of the expression vector pTM1.

Imaging of Viral Particles

Clean coverslips were coated with carbon, hydrophilized, irradiated with ultraviolet light for 12 hours, followed by a glow discharge for a few seconds and then sterilization by dry heat (160° C. overnight). MeWo cells were cultivated on the glass coverslips. When the cells became confluent, they were co-cultivated with either VZV-32 or VZV-MSP infected cells at a 1:8 ratio and incubated at 32° C. At the designated times after infection, the cells were prefixed with 1% glutaradehyde in PBS at 4° C. for 1 hour, rinsed in chilled PBS followed by postfixation with 1% osmium tetraoxide in PBS at 4° C. for 1 hour, and then dehydrated in a graded ethanol series. Finally, after two changes in 100% ethanol, the specimens were subjected to a critical point drying method. In the case of HSV-1 samples, FL cells prepared on coverslips were inoculated with HSV-1 Miyama strain at an MOI of 10. Twenty four hours after virus

inoculation, coverslips were washed with PBS and processed as described for VZV. Subsequently, the specimens were mounted onto aluminum plates and observed with either a Hitachi S-4000 or a Hitachi S-900 SEM. Imaging was performed at the University of Iowa Central Microscopy Research Facility and the University of Wisconsin-Madison Integrated Microscopy Facility.

Results

Analysis of the VZV Isolate by Confocal Microscopy

The virus designated VZV-MSP was initially isolated in human fibroblast monolayers. The cytopathic effect (CPE) was compatible with VZV, but the isolate was poorly reactive with antibodies in a commercial VZV diagnostic kit. Since the isolate did not stain with antibodies to herpes simplex virus (HSV) types 1 and 2 nor did its CPE resemble that of HSV, the virus isolate was further analyzed. When the isolate (VZV passage 1) was received, the infected cell monolayer was trypsin-dispersed and inoculated onto human melanoma cells (VZV passage 2). When CPE was apparent in 5 days, the infected cell monolayer was trypsin-dispersed one more time and inoculated onto 35 mm monolayers for examination by laser scanning confocal microscopy (VZV passage 3). The low passage VZV-32 strain was included in separate dishes as a control virus. When CPE covered about 70% of each monolayer, the infected monolayers were probed with MAb 3B3 against gE and MAb 6B5 against gI and examined by confocal microscopy. In prior studies, it has been shown that these two MAbs do not cross-react with other viral or cellular proteins (Grose, *Annu. Rev. Microbiol.*, 44:59–80 (1990)). As expected from numerous published experiments, MAb 3B3 and MAb 6B5 reacted with the laboratory strain VZV-32. In marked contrast, the anti-gE MAb 3B3 did not attach to cells infected with the VZV-MSP strain, even though MAb 6B5 did bind the infected cells strongly. As an additional control, the anti-gH MAb 206 was added to cultures individually infected with both VZV strains; all VZV-infected cultures were positive in this assay.

The next question addressed was whether the VZV-MSP strain failed to express the entire glycoprotein or whether it has lost an epitope on the glycoprotein. Monoclonal antibodies produced in the epitope-mapping of gE were used. It was previously established that the 3B3 epitope consisted of at least 11 amino acids 151–161 of gE (Duus et al., *J. Virol.*, 70:8961–8971 (1996)). Another monoclonal antibody, MAb 711, attaches to another as yet undefined epitope on the ectodomain of gE. This epitope does not overlap with the 3B3 epitope. Therefore, the above experiment was repeated with MAb 711 as the immunoprobe of VZV-32 and VZV-MSP infected monolayers. Both monolayers stained positively, a result which indicated that gE was expressed in VZV-MSP infected cells but appeared to have lost either a small segment of its ectodomain or just the 3B3 epitope.

Sequence Analysis of VZV-MSP gE

To further investigate the nature of the gE mutation, we used PCR amplification techniques to first determine whether a full-length gE gene (VZV ORF 68) was present in the mutant strain. The full length gene was amplified. Thereafter, primers were used to amplify overlapping portions of the gE gene, each overlapping portion about 300 bases in size, beginning at the upstream region of ORF 68. Each fragment was subjected to DNA sequencing and each sequence was compared with the published Davison and Scott (Davison et al., *J. Gen. Virol.*, 67:1759–1816 (1986)) sequence of the Dumas strain (FIG. **1A**; Genbank Accession number X04370). After analysis of the first 337 codons of VZV-MSP gE ORF, we found the first and most important base change in codon 150 (FIG. **1B**, arrow); the substitution involved a replacement of a guanine by an adenine. Of great interest, this point mutation led to a change in amino acid from aspartic acid to asparagine (FIG. **1B**). Since this alteration in gE occurred one amino acid away from the deduced 3B3 epitope, which is underlined in FIG. **1A**, the sequence data strongly suggested that amino acid 150 was a previously unrecognized contributor to the 3B3 epitope. Further sequencing of VZV-MSP gE revealed one silent mutation in codon 341 of VZV-MSP gE. All other codons were identical to those in the gE sequence of the Dumas strain.

Epitope Mapping of VZV-MSP gE

In an earlier experiment, we had evaluated the 3B3 epitope by inserting the 11-amino-acid sequence into the unrelated VZV ORF 60, namely, the gL glycoprotein (Duus et al., *J. Virol.*, 70:8961–8971 (1996)). The epitope tag within gL was recognized by MAb 3B3 when observed by laser scanning confocal microscopy. To evaluate the contribution of the aspartic acid residue to formation of the epitope, the gL epitope mapping and tagging experiment was repeated in order to insert the aspartic acid residue in its correct location at the N-terminus of the 3B3 epitope. In order to obtain the proper parameters for the mutagenesis primers, one additional codon was inserted along with an aspartic acid (FIG. **1B**). The pTM-1 expression plasmids, including gL-3B3.11 and gL-3B3.13, were transfected into HeLa cells and observed by confocal microscopy after labeling with MAb 3B3. Cells transfected with the gL-3B3.11 plasmid were positive in a restricted cytoplasmic pattern, as previously described by Duus et al., (*J. Virol.*, 70:8961–8971 (1996)). Cells transfected with the gL-3B3.13 were not only more intensely stained, the pattern was more widely distributed throughout the cytoplasm. Cells transfected with the pTM-1 gL plasmid alone were negative.

Subcloning the VZV-MSP gE ORF

After completion of the above experiment, we sought to confirm the epitope experiments by amplifying the entire gE gene from VZV-MSP DNA and inserting it into a pTM-1 expression vector. We had previously cloned wild-type gE into the same expression vector; the same primers were used for the second cloning experiment (Yao et al., *J. Virol.*, 67:305–314 (1993)). After transient transfection with these two forms of VZV gE as well as the pTM-1 vector as a control, the cell lysates were solubilized and subjected to electrophoresis followed by transfer to membranes. Additional control samples for the transfection immunoblotting experiments included Mewo cell monolayers infected with three VZV strains: VZV-32, VZV-Oka and VZV-MSP. Uninfected Mewo cells served as a negative control. All samples were blotted with MAb 3B3 followed by detection using chemiluminesence. The MAb attached to VZV-32, VZV Oka and VZV gE wild type, but not to VZV-MSP, VZV MSP gE or the vector and uninfected cell controls. When VZV-MSP gE was subsequently immunoblotted with a polyclonal monospecific antibody to gE, the result was positive. Thus, these results confirmed that VZV-MSP gE by itself was expressed but lacked the 3B3 epitope.

Alterations in Topography of Egress of Viral Particles

In previously published studies, it was shown that the egress of wild type VZV particles onto the surface of infected cells occurs in a distinctive pattern which was termed "viral highways" (Harson et al., *J. Virol.*, 69:4994–5010 (1995)). The viral highways are composed of thousands of viral particles which emerge in long rows across the surface of the syncytia. When the distribution of

VZV-32 and VZV-MSP particles were compared at a low magnification level by scanning electron microscopy (SEM), wild type virions were again arranged in a pattern consistent with viral highways. Cells infected with the VZV-Oka strain show a similar pattern of viral highways (Grose, *Infect. Dis. Clinics NA*, 10: 489–505 (1996)). In contrast, no such topographical pattern was observed on samples infected with VZV-MSP; instead, viral particles were distributed more uniformly over the cell surface. After observation of numerous monolayers by SEM, it appeared that the number of VZV wild type virions present on the cell surface was less than those of VZV-MSP. The topographical arrangement of VZV-MSP particles also exhibited a high degree of similarity with that of HSV-1, in which thousands of particles covered the cell surface.

In a VZV-infected monolayer, cytopathic effect follows the longitudinal axis of the cells. In VZV-infected human melanoma cell cultures, individual syncytial foci enlarge and eventually merge until the entire monolayer has become a single syncytium. Virions only emerge after syncytia are formed but the virions are never released into the culture medium. If syncytial formation is blocked by adding anti-gH antibody into the culture medium, virions do not egress until the antibody is removed. Based on the imaging studies in this as well as previous reports, it is postulated that virions exit at the leading edge of syncytial foci which are merging. Further, VZV egress (gE/gI mediated) and VZV-induced cell-to-cell fusion (gH/gL mediated) are separate but interdependent events. The mutation on VZV-MSP gE appears to lessen that interdependence.

EXAMPLE 2

Identification of Single Nucleotide Polymorphisms in Other ORFs of VZV-MSP and Assessment of Cell-to-cell Spread

This example provides a more complete characterization of the altered biological properties and genetic composition of this contemporary variant in VZV evolution. For the first time, a varicella zoster virus variant is described which has a cell-to-cell spread phenotype clearly distinguishable from previously characterized varicella zoster virus strains.

Cells, viruses and transfer of infectivity. VZV-MSP was isolated in Minnesota in late 1995. VZV-32 was isolated in Texas in the 1970s (Grose et al., *Infect. Immun.*, 19:199–203 (1978)). Reserve stocks of VZV-32 and VZV-MSP were prepared; thus low passages (<20) were used in all experiments in this report. VZV-Oka was isolated in Japan and attenuated in the 1970s (Takahashi et al., *Biken J.* 18:25–33 (1975)). VZV-Dumas was isolated in Holland and sequenced in its entirety by Davison et al., (Davison et al., *J. Gen. Virol.*, 67:1759–816 (1986)). All strains were propagated in human melanoma cells (MeWo strain). MeWo cells are highly permissive for VZV replication with no release of infectious virus into the culture medium (Grose, *Virology* 101:1–9 (1980)). Transfer of infectivity was carried out by inoculation of trypsin-dispersed infected cells onto an uninfected monolayer at a 1:8 ratio of infected: uninfected cells unless otherwise noted. Similarly, infectious center assays were carried out by described methods; these assays included both melanoma cell and human neonatal foreskin cell substrates (Cole et al., *J. Virol. Methods,* 36:111–8 (1992), Grose et al., *Infect. Immun.*, 19:199–203 (1978)).

Imaging by confocal microscopy. Replicate 35-mm monolayers of MeWo cells were overlaid with VZV-infected cells at a 1:8 ratio of infected:uninfected cells. At 4, 8, 12, 24, and 48 hours post-infection, the monolayers were fixed and permeabilized with 0.5 ml 2% paraformaldehyde with 0.05% Triton X-100. Cells were probed with an anti-IE 62 mouse monoclonal ascites (MAb 5C6, available from C. Grose, University of Iowa, Iowa) at a dilution of 1:1,000 (Ng et al., *J. Virol.*, 68:1350–1359 (1994)). The secondary antibody was goat anti-mouse IgG $F(ab')_2$ conjugated to Alexa 488 at a dilution of 1:2,500 (Molecular Probes, Eugene, Oreg.). Cell nuclei were stained with TOTO-3 (Molecular Probes), a dimeric cyanine nucleic acid stain, at a dilution of 1:10,000. Samples were examined with a BioRad 1024 laser scanning confocal microscope, as described (Duus et al., *Virology* 210:429–440 (1995)).

Quantitative Analysis of Confocal Images. Confocal images were converted to TIFF format images (Confocal Assistant, v. 4.02, Bio-Rad Laboratories (Hercules, Calif.), available from ftp://ftp.genetics.bio-rad.com/Public/confocal/cas) and transferred to a Silicon Graphics Indy workstation in order to produce the color prints by Showcase software program (Silicon Graphics, Mountain View, Calif.)). Confocal images also were analyzed with the Braincox tal_support programs (Frank et al., *Neuroimage*, 5:13–30 (1997)). Similar analyses can be performed with the public domain NIH Image program, which was developed at the U.S. National Institutes of Health (http://rsb.info.nih.gov/nih-image/).

Replication in the SCID-hu mouse. The SCID-hu mouse has been established as an animal model for VZV replication (Moffat et al., *J. Virol.,* 69:5236–42 (1995)). In this model, C.B-17 scid/scid mice were implanted with fetal skin tissue subcutaneously as full thickness dermal grafts. Human fetal tissues were obtained with informed consent according to federal and state regulations and were screened for human immunodeficiency virus. The general care of the experimental animals used for this study was in accordance with the National Institutes of Health guidelines for laboratory animals and in compliance with the Animal Welfare Act (Public Law 94–279) as well as the Stanford University Administrative Panel on Laboratory Animal Care. Animal inoculations were performed according to the previously described protocol (Moffat et al., *J. Virol.*, 69:5236–42 (1995)), viz., an aliquot of infected cell suspension containing 10^5 infectious centers was injected into each implant. Mock-infected implants were injected with human cells alone. Skin implants were harvested at 7, 14 and 21 days post-inoculation. The implants were fixed in 4% paraformaldehyde, paraffin-embedded, cut into 3-μm sections, and stained with hematoxylin and eosin. Tissue sections were examined on a Leitz Diaplan light microscope, and digital images were acquired with an Optronics DEI 750 digital camera (Optronics Engineering, Goleta, Calif.). Digital images were formatted as described above.

Isolation of viral DNA. For all viral strains, a 25 cm^2 monolayer of MeWo cells was infected as described above. After development of 80–100% cytopathology, the infected monolayer was washed thrice with 0.5 ml of 0.01M phosphate buffered saline (PBS), pH 7.4. Infected cells were then harvested by dislodging into 0.5 ml of PBS. Viral DNA was collected with a DNAeasy Kit following the Blood and Body Fluid Protocol (Qiagen Inc, Valencia, Calif.). Following DNAeasy protocol, DNA was placed onto a Microcon 50 filter (Millipore, Bedford, Mass.) and washed twice with 0.5 ml of Nanopure water (Barnstead/Thermolyne, Dubuque, Iowa). Viral DNA was resuspended in 100 μL of Nanopure water. DNA concentration was assessed visually after 1% agarose gel electrophoresis.

PCR amplification and sequencing of VZV genes. For each ORF, a pair of flanking primers was designed to

amplify the gene of interest. PCR amplifications were performed with the Expand High-Fidelity PCR System (Boehringer Mannheim, Indianapolis, Ind.). This system utilizes both Taq DNA and Pwo DNA polymerases, with the 3'–5' proofreading activity of Pwo DNA polymerase allowing increased fidelity (8.5×10^{-6} per bp error rate) (Boehringer Mannheim). After amplification, the PCR product was sequenced by using the dye terminator cycle sequencing chemistry with AmpliTaq DNA polymerase, FS enzyme (Perkin Elmer Applied Biosystems, Foster City, Calif.). Sequencing reactions were performed on and analyzed with an Applied Biosystems Model 373A stretch fluorescent automated sequencer (Perkin Elmer) at the University of Iowa DNA facility. All genes were PCR amplified twice and each PCR fragment was sequenced at least twice to confirm reported mutations. Each DNA sequence was compared to the prototype VZV-Dumas sequence. The accession number for the complete VZV-Dumas sequence is X04370. The primers used for amplification and/or sequencing the amplified fragments are shown in Table 1.

TABLE 1

VZV sequencing and amplification primers

Protein[1]	Primer[2]	bp[3]	Seq/Amp[4]	Sequence[5]	
gB					
(ORF 31)	Scp 1 (S)	-163 to -136	Seq/Amp	GGCGTTTTCATAACCTCCGTTACGGGGG	(SEQ ID NO:8)
	Scp 2 (AS)	2685 to 2658	Seq/Amp	CCCTGTGATGCGTAATGGAGACACATGA	(SEQ ID NO:9)
	Sp 1 (A)	309 to 328	Seq	CTTTGTAATATACCGTCGCC	(SEQ ID NO:10)
	Sp 2 (S)	201 to 220	Seq	CGTACGATTAGAACCAACTC	(SEQ ID NO:11)
	Sp 3 (S)	569 to 588	Seq	GGCATACTACCAATGACACG	(SEQ ID NO:12)
	Sp 4 (S)	929 to 948	Seq	AGTGGCGTGAGGTTGAAGAC	(SEQ ID NO:13)
	Sp 5 (S)	1264 to 1283	Seq	CACCCGACTCGAAATACCAG	(SEQ ID NO:14)
	Sp 6 (S)	1615 to 1634	Seq	TCTGGTAGTACTACGCGTTG	(SEQ ID NO:15)
	Sp 7 (S)	1948 to 1970	Seq	GACTACAGTGAAATTCAACGCCG	(SEQ ID NO:16)
	Sp 8 (S)	2259 to 2279	Seq	CCCGATGAAGGCATTATATCC	(SEQ ID NO:17)
	Sp 9 (A)	1418 to 1437	Amp	TAGCTGGCACCACGACGAGG	(SEQ ID NO:18)
	Sp 10 (A)	2565 to 2584	Amp	TGCGAACACGGGAGTATCCT	(SEQ ID NO:19)
gH					
(ORF 37)	Sp 2 (S)	104 to 131	Seq	CTGCTCTTCTACGAGAATATTCCGACCG	(SEQ ID NO:20)
	Sp 3 (A)	223 to 199	Seq	CGTGTTTTCTATCATTTCCCCAGTG	(SEQ ID NO:21)
	Sp 4 (S)	448 to 471	Seq	ACTACGTTCCCACCAAACCCCCTTG	(SEQ ID NO:22)
	Sp 5 (S)	850 to 873	Seq	GCGGTTACAAGCGACACCACATGG	(SEQ ID NO:23)
	Sp 6 (S)	1165 to 1191	Seq	CTGTTAGATGAGATCGTAGATGTTCAG	(SEQ ID NO:24)
	Sp 7 (S)	1498 to 1514	Seq	GCTACAGAGAGGCAGGCT	(SEQ ID NO:25)
	Sp 8 (S)	1816 to 1840	Seq	TTGCATACCCAACTAGACGAATCTG	(SEQ ID NO:26)
	Sp 9 (S)	2129 to 2152	Seq	TAGAGACGGTCGCACTGCCCCATC	(SEQ ID NO:27)
	Sp 10 (S)	-32 to -5	Seq/Amp	CGGTGATATTGTAGCGCAAGTAACAGC	(SEQ ID NO:28)
	Sp 11 (A)	2605 to 2580	Seq/Amp	CCCAAAGGTAGTGTGTATTATTCGCG	(SEQ ID NO:29)
	Sp 13 (A)	223 to 198	Seq	CGTCTCCTTCGTGTGTTG	(SEQ ID NO:30)
	Sp 14 (A)	1005 to 988	Seq	ATCCAAACTCTCTTCGGG	(SEQ ID NO:31)
	Sp 15 (A)	2218 to 2199	Seq	TCGCCCCCGTGGTTAGATAC	(SEQ ID NO:32)
gE					
(ORF 68)	Ip2 (A)	2061 to 2039	Seq/Amp	CGCTCTAGAACTAGTGGATCCCCCGGGGAATTTGTCACAGGC TTTT	(SEQ ID NO:7)
	NcogpI (S)	1 to 11	Amp	CGACCCGGGGAGCTCCCATGGGGACAGTTAATAAACC	(SEQ ID NO:6)
	Sp 1 (S)	1452 to 1470	Seq	GCATGTTGAAGCCGTAGCA	(SEQ ID NO:33)
	Sp 3 (S)	199 to 217	Seq	ATGCGCGGCTCCGATGGTA	(SEQ ID NO:34)
	Sp 4 (A)	505 to 486	Seq	GGCCTTGGGGTTTTGGATTA	(SEQ ID NO:35)
	Sp 6 (S)	-70 to -48	Seq	GTCCATGGTTTTAGACCTCGGG	(SEQ ID NO:36)
	Sp 7 (S)	543 to 561	Seq	GTTTACTTTACGCGCACCG	(SEQ ID NO:37)
	Sp 8 (S)	823 to 840	Seq	GAATTAGACCCCCCCGAG	(SEQ ID NO:38)
	Sp 9 (S)	1127 to 1146	Seq	TAGAGTGGTTGTATGTCCCC	(SEQ ID NO:39)
	Sp 10 (S)	1601 to 1619	Seq	CACTTCTACGATATGCCGC	(SEQ ID NO:40)
	Sp 12 (A)	846 to 827	Seq	TTCAATCTCGGGGGGGTCTA	(SEQ ID NO:41)
	Sp 13 (A)	1790 to 1772	Seq	TCCGTAGATTCCGAGTCCT	(SEQ ID NO:42)
gL					
(ORF 60)	P1 (S)	-1 to 16	Seq/Amp	CAAGCGCCATGGCATCACATAAAT	(SEQ ID NO:43)
	P2 (A)	913 to 886	Amp	AAACACTAGTCCATGTGCATGTCCCGC	(SEQ ID NO:44)
	Sp 1 (A)	548 to 530	Seq	GCTTGCGGGTTTTTTTGGT	(SEQ ID NO:45)
	Sp 2 (A)	320 to 302	Seq	GCCAGCCCCTTTAAGGTGA	(SEQ ID NO:46)
	Sp 4 (S)	473 to 494	Seq	GCCAATGAAATGAAACTATCGG	(SEQ ID NO:47)
gI					
(ORF 67)	Scp 1 (S)	-60 to -33	Seq/Amp	CGGCTCACAGAGCTGCTCTTCGGTGTAG	(SEQ ID NO:48)
	Scp 2 (A)	1154 to 1137	Seq/Amp	TAATCCTTCCCCTCATATCACAACGCGT	(SEQ ID NO:49)
	Sp 1 (A)	995 to 978	Seq	GCGGCCTCCAACATCACA	(SEQ ID NO:50)
	Sp 2 (A)	383 to 365	Seq	CCAGCATCCGGCTCTGTTG	(SEQ ID NO:51)
	Sp 4 (S)	314 to 337	Seq	CGTGTAGGTACAAACATTCGTGGC	(SEQ ID NO:52)
ORF 47	ScP 1 (S)	-349 to -320	Amp	ATCTAATCCGTGGGGGTGCGAGTGTACAAG	(SEQ ID NO:53)
	ScP 2 (A)	103 to 132	Amp	TCCATGTTCGGTGATGTCTTCTGTAGGCGTG	(SEQ ID NO:54)
	Sp 1 (S)	3 to 22	Seq	GGATGCTGACGACACACCCC	(SEQ ID NO:55)
	Sp 2 (A)	163 to 182	Seq	GTTGCAGTTGACGGATTGGC	(SEQ ID NO:56)
	Sp 3 (S)	341 to 360	Seq	TTGTACCCATCTTCACGCTC	(SEQ ID NO:57)
	Sp 4 (S)	743 to 762	Seq	ATCGAACGTGCGGCCTGACC	(SEQ ID NO:58)
	Sp 5 (S)	1135 to 1154	Seq	CTTCCCGGACAACTGCCCAT	(SEQ ID NO:59)

TABLE 1-continued

VZV sequencing and amplification primers

Protein[1]	Primer[2]	bp[3]	Seq/Amp[4]	Sequence[5]	
IE62 (ORF 62)	Scp 1 (S)	-79 to -50	Seq/Amp	CACCAACCGCAATCGCAATCCTTTGAAGGC	(SEQ ID NO:60)
	Scp 2 (AS)	3990 to 3961	Seq/Amp	TATTAACAACAAACAGTCCGCGCGCCAGTG	(SEQ ID NO:61)
	Sp 1 (S)	318 to 342	Seq	GCCGAGGTCTTCCACACCCGATTCT	(SEQ ID NO:62)
	Sp 2 (S)	749 to 773	Seq	TTTGAAGGTTAAGGTCCCACTCCCG	(SEQ ID NO:63)
	Sp 3 (S)	1142 to 1166	Seq	TACAGGCAGCAGGTCCGGACGCGAA	(SEQ ID NO:64)
	Sp 4 (S)	1511 to 1535	Seq	TTACGAGGCCTCAACGGAACCCGTG	(SEQ ID NO:65)
	Sp 5 (S)	1925 to 1949	Seq	GATCTCCCGCGGTCACCCTTCTCCA	(SEQ ID NO:66)
	Sp 6 (S)	2309 to 2333	Seq	CAAGGCGTACTGTACCCCCGAAACC	(SEQ ID NO:67)
	Sp 7 (S)	2685 to 2709	Seq	ACTCATGCCTGGGCCGGGAACTGGA	(SEQ ID NO:68)
	Sp 8 (S)	3098 to 3122	Seq	CGTCGCATACACCGTGTGTACCCGC	(SEQ ID NO:69)
	Sp 9 (S)	3500 to 3524	Seq	TGCCCTCCCCCCGATTCCCAGAGTA	(SEQ ID NO:70)

[1]The protein that is encoded by the listed ORF.
[2]The designation of the primer.
[3]The base pairs to which the primer hybridizes. The numbering used denotes the transcriptional start site as +1.
[4]Seq, the primer was used as a primer in a sequencing reaction; Amp, the primer was used as a primer in PCR. Some primers were used in both sequencing and amplification.
[5]The nucleotide sequence of the primer, listed 5' to 3'.

Results

Cell-to-cell spread phenotype of VZV-MSP. During the initial assessment of VZV-MSP, it was observed that the egress of VZV-MSP particles in cell culture differed from that of other typical VZV laboratory strains, such as VZV-32 and VZV-Oka. Like other VZV strains, however, infectious virus was not released into the culture medium. The fact that greater numbers of VZV-MSP particles were present on the surface suggested that cell-to-cell spread may be increased. Cell-to-cell spread has been assessed in both herpes simplex virus type 1 (HSV-1) and pseudorabies virus (PRV) through measurement of plaque size in permissive cells or number of cells infected within a typical plaque. Neither technique is easily applicable in the VZV system given the formation of irregularly shaped syncytia by VZV in cell culture and the absence of cell-free virus in tissue culture. Therefore, a technique was developed to assess cell-to-cell spread through confocal microscopic examination.

The spread of low-passage VZV-MSP was compared to the low-passage laboratory strain VZV-32. Human melanoma cells were inoculated with either VZV-32 or VZV-MSP at a 1:8 ratio of infected: uninfected cells. At increasing intervals post-infection, the infected monolayers were probed with an antibody against the VZV immediate early protein 62 (IE 62). The number of cells expressing IE 62 and the intracellular localization of the protein were determined. This assay was based on the observation by Kinchington et al., (*J. Infect. Dis.*, 178 Suppl 1:S16–21 (1998)) that VZV IE 62 is present in the nuclei of infected cells during early stages of infection, but then appears in the cytoplasm during later stages.

At the time point of four hours post-infection, comparing VZV-32 and VZV-MSP by confocal microscopy represents the number of infected cells originally overlaid onto each monolayer. Both monolayers contained similar levels of IE 62 positive cells. At twenty-four hours post-infection the difference in extent of spread between VZV-MSP and VZV-32 was apparent. For VZV-32 at 24 hours, 6–8 cells with advanced infection were present in each focus; IE 62 was present in both nucleus and cytoplasm. A few scattered cells adjacent to the infectious focus contained nuclei with IE 62 concentrated near the membrane; these cells represented a recent transfer of infectivity from the central focus. When the VZV-MSP culture was examined at 24 hours, large syncytia had already formed. A typical syncytium contained 20–30 nuclei, an infectious focus 3 to 4 fold larger than that seen with VZV-32. This experiment was repeated four times with equivalent results.

Quantitative analysis of confocal images of VZV IE 62. To quantify the difference in cell-to-cell spread, multiple confocal images were analyzed with the image analysis programs called Brainvox tal_support programs. Each confocal image is made up of 512×512 pixels, for a total of 262,144 pixels. The green fluorescence channel representing the presence of VZV IE 62 within each confocal image was analyzed. The image analysis program initially assigns a relative signal intensity within each pixel of the confocal image. Then, a threshold of signal intensity is calculated to remove background signals. This analysis facilitated quantification of all pixels within each confocal image that contained IE 62. Thus, confocal microscopy of IE 62 coupled with image analysis facilitated a comparison of the extent of viral spread between two different VZV strains.

The results for this analysis are shown in FIG. **3**. There was no major difference between the extent of IE 62 spread for VZV-32 and VZV-MSP at 4, 8, and 12 hours post-infection. The results at both 4 and 8 hours, in particular, demonstrated that each monolayer was infected with a similar inoculum of infected cells. Also, the lack of a difference between the three time points (4 hours–12 hours) confirmed that the replication cycle for both VZV strains was greater than 12 hours, in agreement with previous studies. However, at twenty-four hours post-infection, there was a noticeable difference between spread of VZV-MSP and VZV-32. The extent of VZV-MSP spread was at least two-fold greater than VZV-32. At forty-eight hours post-infection, this difference increased further, as VZV-MSP spread was four-fold greater than VZV-32. Thus, image analysis provided a new method by which to measure differences in cell-to-cell spread between VZV strains. Again, this methodology is particularly suited for VZV because cell free virus is not released spontaneously from infected cell cultures; even after sonic disruption of infected monolayers, most viral particles remain attached to remnants of outer cellular membranes.

VZV infectious center assays. Because the results in the previous experiments represented a new application of confocal microscopy, a traditional method was also used to

confirm the differences in VZV cell-to-cell spread, namely, infectious center assays. For these titrations, the initial virus inocula were replicate samples of VZV-infected cells frozen and stored in liquid nitrogen. After freezing, one aliquot was thawed and titrated from each lot. The inoculum for each 35-mm tissue culture dish was 500 infectious centers. Two dishes were harvested and assayed at each of the following time points: immediately after inoculation (0 hour), 24 hours, and 48 hours post-inoculation (FIG. **4**). When comparing the average fold increase of VZV-MSP infectious centers to VZV-32 infectious centers, the spread of VZV-MSP was consistently greater than the spread of VZV-32 over both the first 24 hour period (24 hr pi) and the second 24 hr period (48 hr pi). Otherwise stated, at 48 hours post-infection, the cytopathic effect of VZV-MSP was complete, while numerous infectious center titrations with VZV-32 demonstrated that a 60–72 hr interval was required for similar spread (Grose et al., *Infect. Immun.*, 19:199–203 (1978)). Furthermore, the rapidity of VZV-32 spread was not altered over the initial 20 passages. VZV-Oka and VZV-Ellen titrations exhibited a time course similar to that of VZV-32. Therefore, results from both quantitative confocal microscopic image analyses and infectious center assays documented that the spread of VZV-MSP was 3–4 fold greater than the spread of VZV-32.

Growth of VZV-MSP in the SCID-hu mouse. The SCID-hu mouse has provided the first reproducible animal model of VZV pathogenesis. Published studies have documented the pathology of viral infection in human thymus/liver and skin implants after inoculation with parental and vaccine Oka strains as well as low passage wild type virus (Moffat et al., *J. Virol.*, 69:5236–42 (1995), Moffat et al., *J. Virol.*, 72:965–974 (1998)). To assess whether VZV-MSP showed enhanced pathology in the SCID-hu mouse model, skin implants infected with VZV-MSP were harvested at 7, 14, and 21 days after infection. At 7 days post-infection, numerous foci of infection were visible in the epidermis of the human skin implant. By 14 days the foci coalesced into large necrotic lesions with histopathology typical of varicella vesicles. These vesicles were characterized by epidermal hyperplasia, balloon cells, and the separation of the keratin roof from the epidermis. After 21 days, the infection had spread into the dermis and destroyed the entire implant. Samples of the implants also were examined by previously described electron microscopy methods; the virion formation closely resembled that shown in FIG. **2** of the report by Moffat et al., (*J. Virol.*, 72:965–974 (1998)). Mock infected skin implants showed normal skin structure consisting of a thin layer of keratinocytes above the dermis and hair follicles.

Prior published studies had not shown such a rapid progression of pathology in the SCID-hu mouse infected with VZV. To further assess this aspect of VZV infection in the animal model, another experiment was performed with a clinical isolate passaged even fewer times than VZV-32; in addition, the low passage parent VZV-Oka strain was included. Again, the skin samples were collected and examined at days 7, 14, and 21 post-inoculation. When all the specimens were reviewed, the histopathology of the clinical isolate and parental VZV-Oka at 21 days post-inoculation were similar, and for both strains the histopathology was approaching that caused by VZV-MSP at 14 days post-inoculation. Even at 21 days, however, the former two viral strains never caused the total destruction seen after VZV-MSP infection. In short, the progression of VZV-MSP through the skin implant was noticeably more extensive than seen with other viral strains tested in the SCID-hu animal model.

Genetic analysis of other major glycoproteins of VZV-MSP. After documenting the enhanced cell-to-cell spread of VZV-MSP, it was determined whether mutations were present in ORFs other than gE that may be contributing to this phenotype. Specifically, ORFs 31, 37, 60, and 67 coding for VZV gB, gH, gL, and gI, respectively, were analyzed. The ORFs were amplified from the VZV-MSP viral genome and sequenced, then compared to the published VZV-Dumas sequence. Neither gI nor gL gene contained any nucleotide differences when compared to the nucleotide sequences of VZV-Dumas. Further, the gB sequence was identical to that of VZV-Dumas. However, VZV-MSP gH contained a single point mutation within codon 269 (C<u>C</u>A→C<u>T</u>A), converting a proline residue in the predicted VZV-Dumas peptide sequence to a leucine residue in VZV-MSP gH.

Given the presence of mutations within VZV-MSP gE and gH, a similar genetic analyses of VZV-32 was performed. As expected, VZV-32 lacked the D150N mutation within gE. VZV-32 gH, however, revealed the identical point mutation found within codon 269 of VZV-MSP gH. Thus, the mutation within VZV-MSP gH cannot account for the VZV-MSP cell-spread phenotype. VZV-32 contained one additional mutation within ORF 67 (gI) which would lead to a Q5H substitution (CA<u>A</u>→CA<u>T</u>). This substitution was within the probable leader sequence of VZV gI and thus would not be present in mature gI (Davison et al., *J. Gen. Virol.*, 67:1759–816 (1986)). Altogether, within five major glycoprotein ORFs, VZV-MSP contained two point mutations which caused amino acid substitutions when compared to VZV-Dumas: D150N in gE, and P269L in gH (FIG. **5**).

In addition to 5 ORFs, we sequenced major portions of the 5' untranslated regions of ORFs 31, 60, 67 and 68. All regions were identical to VZV-Dumas except for that of ORF 60. The latter region contained four polymorphisms; these ranged from 554 to 1320 nucleotides from the ORF 60 initiation codon (FIG. **5**). It is very unlikely that these polymorphisms will alter the expression of gL since they are located over 500 nucleotides upstream of the gL start site.

Genetic analysis of VZV-MSP regulatory proteins and kinases. Although viral glycoproteins are the most likely candidates for mediating the cell-to-cell spread phenotype of VZV-MSP, we considered the possibility that an alteration in immediate early (IE) regulatory events may contribute to this enhanced cell-to-cell spread phenotype. VZV expresses one predominant species, IE 62, which acts as the major regulatory protein for viral gene expression. This protein contains a potent acidic activation domain at its N-terminus and is a component of the virus particle. Therefore, the IE 62 gene of VZV-MSP was sequenced, but detected only one silent polymorphism within codon 30 when compared to the Dumas strain (GC<u>G</u>→GC<u>C</u>) (FIG. **5**). Thus, the peptide sequence of VZV-MSP IE 62 was identical to the predicted VZV-Dumas sequence. Further, we sequenced the 5' untranslated region containing 525 nucleotides and this region was identical to VZV-Dumas. In addition, we sequenced the adjacent VZV-MSP ORF 61, which encodes the functional homolog of HSV-1 ICP0. Again, the nucleotide sequence was identical to VZV-Dumas.

Previous studies have shown that the viral protein kinase VZV ORF 47 can phosphorylate IE 62 (Ng, et al., 1994). Also, VZV ORF 66 encodes a protein kinase which has been shown to affect the intracellular localization and transactivation function of IE 62. Based upon these results, we wanted to determine whether mutations in these viral kinases could affect the function of IE 62 within VZV-MSP infected cells. Therefore, we sequenced both protein kinase genes within the VZV-MSP genome and found both to be

identical to the prototype VZV-Dumas sequence (FIG. **5**). Thus, there was no genetic evidence of polymorphisms within either of two regulatory ORFs or either of two viral protein kinase ORFs. In short, after sequence analysis of over 15% of the VZV-MSP genome, the main impression was a striking similarity with VZV-Dumas except for the notable exceptions mentioned earlier.

EXAMPLE 3

Single Nucleotide Polymorphisms in Major Open Reading Frames of Other Varicella Zoster Viruses

Materials and Methods

Viruses. VZV-MSP was isolated in Minnesota in late 1995. VZV-32 was isolated in Texas in the 1970s. VZV-Oka was isolated in Japan and attenuated in the 1970s. VZV-VSD was a wildtype virus collected in South Dakota in the 1980s. VZV-VIA was isolated in Iowa from a child with chickenpox in the 1990s. VZV-Iceland was isolated in Iceland from vesicle fluid of a child with chickenpox in the 1990s. VZV-Ellen was originally isolated in Georgia from a child with chickenpox in the 1960s and obtained from the American Type Culture Collection. VZV 80-2 was originally isolated in Pennsylvania from an adult with herpes zoster in the 1980s.

Propagation of viruses. All viral strains except VZV 80-2 virus were propagated in human melanoma cells (MeWo strain). Transfer of infectivity was performed by inoculation of trypsin dispersed infected cells onto an uninfected monolayer at a 1:8 ratio. Each 25 cm^2 VZV-infected monolayer was allowed to incubate until cytopathology reached 80%. The monolayer was then washed thrice with 5 ml of 0.01M phosphate buffered saline (PBS) of pH 7.4. Cells were dislodged by scraping into 0.5 ml PBS. Viral DNA was collected from the cells using the DNeasy Tissue Kit following the DNeasy Protocol for Cultured Animal Cells (Qiagen Inc). Collected DNA was cleaned by placing on a Microcon 50 filter and washing twice with 0.5 ml of Nanopure water. (Barnstead/Thermolyne). Viral DNA was resuspended in 100 μl of Nanopure water. The VZV 80-2 viral genome DNA was present in two cloned restriction enzyme libraries prepared by Ecker et al., (*Proc. Natl. Acad. Sci. USA*, 79:156–160 (1982)).

PCR amplification and sequencing of viral DNA. PCR amplification was performed with primers flanking the region of interest (Table 1). The Expand High Fidelity PCR System was used in the PCR amplification procedure (Roche Molecular Biochemicals). This system includes Taq DNA and Pwo DNA polymerases, with the 3'–5' proofreading activity of the Pwo DNA polymerase to increase the fidelity (Roche Molecular Biochemicals). Electrophoresis of each sample was carried out in a 1% agarose gel to determine concentration. The DNA sequencing reactions were performed using dye terminator cycle sequencing chemistry with AmpliTaq DNA polymerase, FS enzyme (PE Applied Biosystems, Foster City, Calif.). Reactions were run and subsequently analyzed with an Applied Biosystems Model 373A stretch fluorescent automated sequencer at the University of Iowa DNA Facility. Sequences were further analyzed using the program DNASIS V2.0 (Hitachi Software Engineering Co.). Any region of a VZV genome which differed in sequence from that of the prototypic VZV Dumas was re-amplified in a second PCR step and subjected to a second sequencing analysis.

Results

Polymorphisms in the VZV gE Gene

The VZV gE gene was of greatest interest because of the discovery of the gE mutant strain VZV-MSP. Surprisingly, six gE polymorphisms were found among the eight tested strains and isolates, four of which caused amino acid substitutions (FIG. **6**). However, none of the tested strains contained the D150N mutation within the 3B3 epitope of VZV-MSP. VZV-Ellen, VZV-Iceland, and VZV 80-2 had three identical polymorphisms. One was a synonymous mutation within codon 220. Two non-synonymous mutations in these three strains caused amino acid substitutions within codons 40 (T→I) and 536 (L→I). The vaccine strain VZV-Oka also contained the mutation within codon 40, but lacked the other two mutations found within VZV-Ellen, VZV-Iceland, and VZV 80-2. VZV-VSD was the only strain tested which contained a polymorphism within the gE cytoplasmic domain of gE. Interestingly, this change within codon 603 (G→D) inserted an additional acidic amino acid adjacent to the acidic casein kinase II phosphorylation site of gE. VZV-32 and VZV-VIA were the only strains tested that did not contain gE substitutions when compared to the Dumas strain. Since the mutations previously found in VZV-MSP gE were not discovered in any other strain, VZV-MSP gE retained a unique sequence among all currently tested strains and isolates.

Polymorphisms in the VZV gI Gene

The discovery of several polymorphisms in the gE gene of the 8 strains was unexpected. Since VZV gE and gI proteins are commonly found in a complex in the infected cell culture, the gI gene was the next obvious candidate for further genetic analysis. Sequencing of ORF 67 led to the discovery of two changes from the published Dumas sequence (FIG. **7**). VZV-32 had an A to C substitution at bp 15 of the ORF that resulted in a glutamine to histidine substitution. VZV Oka also had a silent change of G to A at bp 546. The number of gI sequence variants was less than that seen with gE and may suggest that gI function requires a more rigid amino acid sequence.

Polymorphisms in the VZV gH Gene

Next to the VZV gE:gI complex, the gH:gI complex has been most extensively studied because of its role in cell-to-cell fusion. Overall, the eight strains contained nine polymorphisms, three of which caused amino acid substitutions (FIG. **8**). Again, VZV-Ellen, VZV-Iceland, and VZV 80-2 were remarkably similar, with identical changes within codons 76 (R→K) and 700 (R→K) as well as silent substitutions within codons 13, 676 and 727. VZV-Ellen possessed a unique polymorphism within codon 418 that allowed differentiation from VZV-Iceland and VZV 80-2. VZV-32 and VZV-VIA both contained a silent change within codon 815 not present in any other tested strain. VZV-MSP contained only the P269L polymorphism shared by seven strains, including VZV-32.

Polymorphisms in the VZV gL Gene

In a manner similar to gE and gI, gL is invariably linked with the gH protein in VZV-infected cultures. For this reason, the gL ORF was a another candidate gene for sequence analysis. The result was striking: only VZV-Oka gL gene differed from prototype Dumas gL gene (FIG. **9**). The first change included the insertion of a methionine codon between amino acids 9 and 10. Secondly, there was a G to A substitution at bp 320 of VZV-Dumas that resulted in a glycine to aspartic acid change in the protein. The fact that the gL genes from the 7 other strains were identical to the prototype gL gene may suggest, as with the gI protein, that gL function requires a protein that is restricted in its genetic variability.

Polymorphisms in the VZV gB Gene

The gB gene is one of the most conserved genes among all herpesviruses. In the case of VZV, this protein is impor-

tant in the infectious cycle of the virus, based on the previous evidence that addition of anti-gB monoclonal antibody to an infected culture inhibits the progression of infection (Montalvo et al., *J. Virol.*, 61:2877–2884 (1987)). Of the eight strains in which gB was sequenced, 7 were identical to the prototype Dumas gB gene. As with the gL gene, only the Oka gB gene was different from Dumas. The Oka gB had three alterations: an A to C change at bp 217, which led to an amino acid substitution of a threonine to a proline at residue 73; a G to T change at bp 391 resulting in a aspartic acid to a tyrosine substitution at amino acid 131; finally, a silent change of A to C at bp 294. In short, the sequence of gB was highly constrained.

Polymorphisms in the VZV IE62 Gene

The fact that polymorphisms were easily discernible in the major glycoprotein structural genes led to examining whether a similar situation existed in the major VZV regulatory gene called IE62. Overall, 38 polymorphisms were found in the IE 62 gene of the eight tested strains, when compared to the VZV-Dumas IE 62 gene (FIG. **10**). As was the case with the gE and gH gene sequences, VZV-Ellen, VZV-Iceland, and VZV 80-2 showed remarkable similarity in their IE 62 genes. Identical silent substitutions within IE 62 were present in these strains at codons 61, 129, and 1071. Also, these three strains contained an identical polymorphism at codon 703 (V→A). VZV-Oka was similar to these three strains, as it shared the silent IE 62 substitutions within codons 61 and 129, but lacked the change at codon 1071. VZV-Oka shared additional polymorphisms with VZV-Ellen at codons 341 and 958 (R→G) that were not present in VZV-Iceland and VZV 80-2. VZV-Ellen revealed nine unique substitutions in addition to those shared with VZV-Iceland, VZV 80-2 and VZV-Oka.

VZV-32 and VZV-VIA both contained identical polymorphisms within IE 62 at codons 516, 1057 (Q→R), 1072 (Q→R), 1080, and 1241. VZV-32 also contained five unique changes as well as containing the R958G mutation found in VZV-Ellen and VZV-Oka. VZV-VIA revealed four unique polymorphisms, which combined with the unique changes within VZV-32 allow differentiation of VZV-32 and VZV-VIA. VZV-VSD contained one unique substitution (A→V at codon 602) but otherwise was identical to the Dumas strain. Overall, numerous mutations were found within IE 62 among the tested VZV strains. However, VZV-MSP IE 62 contained no polymorphisms, other than the synonymous substitution found within codon 30, when compared to the Dumas strain. Thus, there was no genetic evidence that IE62 contributed to the VZV-MSP phenotype

Polymorphisms in the VZV Protein Kinase Gene

The VZV genome encodes at least two putative protein kinases, one in the UL region (ORF 47) and the second in the US region of the genome (ORF 66). The ORF 47 kinase is known to phosphorylate the IE62 gene product. Sequencing of the ORF47 protein revealed two variations from Dumas. VZV Ellen, Oka and Iceland shared a silent A to G transition at nucleotide 1449. VZV Ellen had a unique transversion of T to C at nucleotide 913, which caused an amino acid change from serine to proline. The VZV 80-2 gene 47 was not sequenced because neither cloned VZV DNA library contained a complete ORF (Ecker et al., *Proc. Natl. Acad. Sci. USA*, 79:156–160 (1982)).

Transitions Versus Transversions

Mutations that result in the substitution of a pyrimidine for a pyrimidine or a purine for a purine are called transitions. Substitutions of a purine for a pyrimidine and vice versa are transversions. Transversions are much less common than transitions in the human genome. When the number of transitions and transversions were counted for the sequenced VZV genes, 78% were transitions. Interestingly, all three substitutions in gB were transversions. This result was in contrast with the other genes. The gE gene had 1 transversion and 5 transitions. The gH gene had 3 transversions and 6 transitions. The gI gene had 1 transversion and 1 transition. The gL gene had 6 transitions. The ORF 47 gene had 2 transitions. The IE62 gene had 5 transversions and 33 transitions. Even though there were many polymorphisms in some IE62 genes, the fact that the IE62 gene of the VZV-MSP strain differed by only one transversion from VZV-Dumas should be noted.

The complete disclosure of all patents, patent applications, and publications, and electronically available material (e.g., GenBank amino acid and nucleotide sequence submissions) cited herein are incorporated by reference. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

Sequence Listing Free Text	
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SEQUENCE LISTING

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<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 6

cgacccgggg agctcccatg gggacagtta ataaacc 37

<210> SEQ ID NO 7
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 7

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<210> SEQ ID NO 8
<211> LENGTH: 28

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<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
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<400> SEQUENCE: 8

ggcgttttca taacctccgt tacggggg 28

<210> SEQ ID NO 9
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 9

ccctgtgatg cgtaatggag acacatga 28

<210> SEQ ID NO 10
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<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 10

ctttgtaata taccgtcgcc 20

<210> SEQ ID NO 11
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<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 11

cgtacgatta gaaccaactc 20

<210> SEQ ID NO 12
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 12

ggcatactac caatgacacg 20

<210> SEQ ID NO 13
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 13

agtggcgtga ggttgaagac 20

<210> SEQ ID NO 14
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

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<400> SEQUENCE: 14

cacccgactc gaaataccag 20

<210> SEQ ID NO 15
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<400> SEQUENCE: 15

tctggtagta ctacgcgttg 20

<210> SEQ ID NO 16
<211> LENGTH: 23
<212> TYPE: DNA
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gactacagtg aaattcaacg ccg 23

<210> SEQ ID NO 17
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<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
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<400> SEQUENCE: 17

cccgatgaag gcattatatc c 21

<210> SEQ ID NO 18
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
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<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 18

tagctggcac cacgacgagg 20

<210> SEQ ID NO 19
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<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
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<400> SEQUENCE: 19

tgcgaacacg ggagtatcct 20

<210> SEQ ID NO 20
<211> LENGTH: 28
<212> TYPE: DNA
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<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 20

ctgctcttct acgagaatat tccgaccg 28

<210> SEQ ID NO 21
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<212> TYPE: DNA
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<400> SEQUENCE: 21

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<211> LENGTH: 24
<212> TYPE: DNA
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<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 23

gcggttacaa gcgacaccac atgg 24

<210> SEQ ID NO 24
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 24

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<210> SEQ ID NO 25
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 25

gctacagaga ggcaggct 18

<210> SEQ ID NO 26
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 26

ttgcataccc aactagacga atctg 25

<210> SEQ ID NO 27
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

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<400> SEQUENCE: 27

tagagacggt cgcactgccc catc 24

<210> SEQ ID NO 28
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<400> SEQUENCE: 28

cggtgatatt gtagcgcaag taacagc 27

<210> SEQ ID NO 29
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 29

cccaaaggta gtgtgtatta ttcgcg 26

<210> SEQ ID NO 30
<211> LENGTH: 18
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<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 30

cgtctccttc gtgtgttg 18

<210> SEQ ID NO 31
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<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 31

atccaaactc tcttcggg 18

<210> SEQ ID NO 32
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 32

tcgcccccgt ggttagatac 20

<210> SEQ ID NO 33
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<212> TYPE: DNA
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<400> SEQUENCE: 33

gcatgttgaa gccgtagca 19

<210> SEQ ID NO 34
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<212> TYPE: DNA
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<400> SEQUENCE: 34

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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 35

ggccttgggg ttttggatta 20

<210> SEQ ID NO 36
<211> LENGTH: 22
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 36

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<210> SEQ ID NO 37
<211> LENGTH: 19
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 37

gtttacttta cgcgcaccg 19

<210> SEQ ID NO 38
<211> LENGTH: 18
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 38

gaattagacc cccccgag 18

<210> SEQ ID NO 39
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 39

tagagtggtt gtatgtcccc 20

<210> SEQ ID NO 40
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

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<400> SEQUENCE: 40

cacttctacg atatgccgc 19

<210> SEQ ID NO 41
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 41

ttcaatctcg ggggggtcta 20

<210> SEQ ID NO 42
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<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 42

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<210> SEQ ID NO 43
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 43

caagcgccat ggcatcacat aaat 24

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<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
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<400> SEQUENCE: 44

aaacactagt ccatgtgcat gtcccgc 27

<210> SEQ ID NO 45
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 45

gcttgcgggt ttttttggt 19

<210> SEQ ID NO 46
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 46

gccagcccct ttaaggtga 19

<210> SEQ ID NO 47
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<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 47

gccaatgaaa tgaaactatc gg 22

<210> SEQ ID NO 48
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 48

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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 49

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<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 50

gcggcctcca acatcaca 18

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<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
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<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 51

ccagcatccg gctctgttg 19

<210> SEQ ID NO 52
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 52

cgtgtaggta caaacattcg tggc 24

<210> SEQ ID NO 53
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

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<400> SEQUENCE: 53

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<210> SEQ ID NO 54
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 54

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<210> SEQ ID NO 55
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 55

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<210> SEQ ID NO 56
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
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<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 56

gttgcagttg acggattggc 20

<210> SEQ ID NO 57
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 57
ttgtacccat cttcacgctc 20

<210> SEQ ID NO 58
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 58

atcgaacgtg cggcctgacc 20

<210> SEQ ID NO 59
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 59

cttcccggac aactgcccat 20

<210> SEQ ID NO 60
<211> LENGTH: 30

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<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 60

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<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 61

tattaacaac aaacagtccg cgcgccagtg 30

<210> SEQ ID NO 62
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 62

gccgaggtct tccacacccg attct 25

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<211> LENGTH: 25
<212> TYPE: DNA
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<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 63

tttgaaggtt aaggtcccac tcccg 25

<210> SEQ ID NO 64
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 64

tacaggcagc aggtccggac gcgaa 25

<210> SEQ ID NO 65
<211> LENGTH: 25
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<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 65

ttacgaggcc tcaacggaac ccgtg 25

<210> SEQ ID NO 66
<211> LENGTH: 25
<212> TYPE: DNA
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<223> OTHER INFORMATION: Oligonucleotide primer

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<400> SEQUENCE: 66

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<210> SEQ ID NO 67
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 67

caaggcgtac tgtacccccg aaacc 25

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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 68

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<210> SEQ ID NO 69
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<212> TYPE: DNA
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<400> SEQUENCE: 69

cgtcgcatac accgtgtgta cccgc 25

<210> SEQ ID NO 70
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 70

tgccctcccc ccgattccca gagta 25

<210> SEQ ID NO 71
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: ARTIFICIAL
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 71

aagctccaag tctcggtgta cc 22

<210> SEQ ID NO 72
<211> LENGTH: 350
<212> TYPE: PRT
<213> ORGANISM: VARICELLA ZOSTER

<400> SEQUENCE: 72

Met Gly Thr Val Asn Lys Pro Val Val Gly Val Leu Met Gly Phe Gly
1 5 10 15

Ile Ile Thr Gly Thr Leu Arg Ile Thr Asn Pro Val Arg Ala Ser Val
20 25 30

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Leu Arg Tyr Asp Asp Phe His Thr Asp Glu Asp Lys Leu Asp Thr Asn
        35                  40                  45

Ser Val Tyr Glu Pro Tyr Tyr His Ser Asp His Ala Glu Ser Ser Trp
    50                  55                  60

Val Asn Arg Gly Glu Ser Ser Arg Lys Ala Tyr Asp His Asn Ser Pro
65                  70                  75                  80

Tyr Ile Trp Pro Arg Asn Asp Tyr Asp Gly Phe Leu Glu Asn Ala His
                85                  90                  95

Glu His His Gly Val Tyr Asn Gln Gly Arg Gly Ile Asp Ser Gly Glu
            100                 105                 110

Arg Leu Met Gln Pro Thr Gln Met Ser Ala Gln Glu Asp Leu Gly Asp
        115                 120                 125

Asp Thr Gly Ile His Val Ile Pro Thr Leu Asn Gly Asp Asp Arg His
    130                 135                 140

Lys Ile Val Asn Val Asp Gln Arg Gln Tyr Gly Asp Val Phe Lys Gly
145                 150                 155                 160

Asp Leu Asn Pro Lys Pro Gln Gly Gln Arg Leu Ile Glu Val Ser Val
                165                 170                 175

Glu Glu Asn His Pro Phe Thr Leu Arg Ala Pro Ile Gln Arg Ile Tyr
            180                 185                 190

Gly Val Arg Tyr Thr Glu Thr Trp Ser Phe Leu Pro Ser Leu Thr Cys
        195                 200                 205

Thr Gly Asp Ala Ala Pro Ala Ile Gln His Ile Cys Leu Lys His Thr
    210                 215                 220

Thr Cys Phe Gln Asp Val Val Val Asp Val Asp Cys Ala Glu Asn Thr
225                 230                 235                 240

Lys Glu Asp Gln Leu Ala Glu Ile Ser Tyr Arg Phe Gln Gly Lys Lys
                245                 250                 255

Glu Ala Asp Gln Pro Trp Ile Val Val Asn Thr Ser Thr Leu Phe Asp
            260                 265                 270

Glu Leu Glu Leu Asp Pro Pro Glu Ile Glu Pro Gly Val Leu Lys Val
        275                 280                 285

Leu Arg Thr Glu Lys Gln Tyr Leu Gly Val Tyr Ile Trp Asn Met Arg
    290                 295                 300

Gly Ser Asp Gly Thr Ser Thr Tyr Ala Thr Phe Leu Val Thr Trp Lys
305                 310                 315                 320

Gly Asp Glu Lys Thr Arg Asn Pro Thr Pro Ala Val Thr Pro Gln Pro
                325                 330                 335

Arg Gly Ala Glu Phe His Met Trp Asn Tyr His Ser His Val
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<210> SEQ ID NO 73
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: VARICELLA ZOSTER

<400> SEQUENCE: 73

Val Asp Gln Arg Gln Tyr Gly Asp Val Phe Lys Gly Asp
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<210> SEQ ID NO 74
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: VARICELLA ZOSTER
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<400> SEQUENCE: 74

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<210> SEQ ID NO 75
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: VARICELLA ZOSTER

<400> SEQUENCE: 75

gtgaaccaac gtcaatacgg tgacgtgttt aaaggagat 39

<210> SEQ ID NO 76
<211> LENGTH: 124884
<212> TYPE: DNA
<213> ORGANISM: Varicella zoster

<400> SEQUENCE: 76

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agcctttaac aaaacccgcg ccttttgcgt ccacccctcg tttactgctc ggatggcgac 180

cgtgcactac tcccgccgac ctgggacccc gccggtcacc ctcacgtcgt cccccagcat 240

ggatgacgtt gcgacccccа tcccctacct acccacatac gccgaggccg tggcagacgc 300

gccccccccc tacagaagcc gcgagagtct ggtgttctcc ccgcctcttt ttcctcacgt 360

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cattgcctgt cacgtgaaaa gactgtgtta cccaataaag gttctacaaa aaatgcttta 1080

ttgggtgttt gtttaatagc tattatcgta acccaccccc gtaaaatcat aaaatgcatg 1140

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tgcgtcccag tttaaacgcc accgccgagg aaaatcccgc gtcagaaacg cgatgtttat 1260

tacgagtgct tgcggggaga actgtagacc tgccaggcgg aggaacgtta cacattacct 1320

gtaccaaaac ctatgtaatt attggcaaat atagcaaacc cggcgaacgt cttagccttg 1380

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ctccatctat gggtacattt ctccgcacac acggtttaag taacagagat ctctgtttat 1560

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aaaataccac tgaaacacct tgtataacgt cgacgttaac atgcaacttg acagaagact 1680

ctggtgaagc cgcacttacc acgtcagacc gacccactct cccaacccta acagcccaag 1740

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aaatacacct tttttcgatt gtacgtattt ttatttaaat gtgtagttca tagtccgccg 1920

acagccgctc gggcttttcc cccacataca acatgatcgt atgcctcgga tgcaccggtc 1980

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What is claimed is:

1. A method for detecting antibodies that specifically bind to a varicella zoster polypeptide, the method comprising:

contacting a biological sample with a preparation to form a mixture, wherein the biological sample comprises an antibody and the preparation comprises a varicella zoster polypeptide that is encoded by a polymorphism of ORF68 and that is not bound by monoclonal antibody 3B3;

incubating the mixture under conditions to allow the antibody to specifically bind the polypeptide to form a polypeptide:antibody complex; and

detecting the presence or absence of the polypeptide:antibody complex, wherein detecting the polypeptide:antibody complex indicates the presence of antibodies that specifically bind to a varicella zoster polypeptide.

2. The method of claim 1 wherein the preparation comprises whole varicella zoster virus that expresses the varicella zoster polypeptide.

3. The method of claim 2 wherein the whole varicella zoster virus is VZV-MSP.

4. The method of claim 1 wherein the varicella zoster polypeptide in the preparation is an isolated varicella zoster polypeptide or fragment thereof.

5. The method of claim 1 wherein the biological sample is selected from the group consisting of blood, vesicle fluid, bone marrow, brain tissue, and combinations thereof.

6. The method of claim 1 wherein the varicella zoster polypeptide is encoded by a nucleotide sequence corresponding to nucleotides 115,808 to 117,679 of SEQ ID NO: 76, wherein the nucleotide at position 116,255 is an adenine.

7. The method of claim 2 wherein the whole varicella zoster virus is a modified varicella zoster virus, the virus having the ATCC designation VR-795 wherein the nucleotide sequence of the virus comprises a polymorphism of ORF68.

8. A kit for detecting antibodies that specifically bind to a varicella zoster polypeptide comprising a whole varicella zoster virus of claim 2.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,843,997 B2
APPLICATION NO. : 10/288823
DATED : January 18, 2005
INVENTOR(S) : Charles F. Grose et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On The Title Page Item (56), delete "6,087,170 A 7/2000 Kemble" and insert -- 6,087,170 A 6/2000 Kemble --; and

On The Title Page Item (56) Other Publications (first column), delete "ATCC CCL-171, "Homo sapiens (human," [online]. Retrieved on May 16, 2001. Retrieved from the Internet; <URL:http://phage.atcc.org/cgi-bin/searchengine/longview.cgi!view=ce, 317407, CCL-171&ctext-CCL.-1,3 pages." and insert -- ATCC CCL-171, "Homo sapiens (human," [online]. Retrieved on May 16, 2001. Retrieved from the Internet; <URL:http://phage.atcc.org/cgi-bin/searchengine/longview.cgi?view=ce, 317407, CCL-171&text-CCL.-1,3 pages. --; and

On The Title Page Item (56), under Other Publications (second column), delete "ATCC VR-586, "Variella-Zoster," [online]. Retrieved on June 5, 2001. Retrieved from the Internet <URL:http//phage.atcc.org/cgi-bin/searchengine/longview.cgi!view-av, 343894, VR-586&text=VR-586>, 2 pages." and insert -- ATCC VR-586, "Varicella-Zoster," [online]. Retrieved on June 5, 2001. Retrieved from the Internet <URL:http//phage.atcc.org/cgi-bin/searchengine/longview.cgi?view-av, 343894, VR-586&text=VR-586>, 2 pages. --; and

On The Title Page Item (56), under Other Publications (second column), delete "ATCC VR-785, "Varicella-Zoster deposited as Varicella," [online]. Retrieved on May 16, 2001. Retrieved from the Internet:<URL:Http://phage.atcc.org/cgi-bin/searchengine/longview.cgi!view-av,476976, VR-795&text=VR-7.9>, 2 pages." and insert -- ATCC VR-785, "Varicella-Zoster deposited as Varicella," [online]. Retrieved on May 16, 2001. Retrieved from the Internet:<URL:Http://phage.atcc.org/cgi-bin/searchengine/longview.cgi?view-av,476976,VR-795&text=VR-7.9>, 2 pages. --; and

On The Title Page, Item (56) under Other Publications (second column), delete "ATCC VR-916, "Varicella-Zoster deposited as Varicella," [online]. Retrieved on Sept. 6, 200. Retrieved from the Internet:<URL:Http://phage.atcc.org/cgi-bin/searchengine/longview.cgi!view-av,554286&text=varicella>, 1 page." and insert -- ATCC VR-916, "Varicella-Zoster deposited as Varicella," [online]. Retrieved on Sept. 6, 200. Retrieved from the Internet:<URL:Http://phage.atcc.org/cgi-bin/searchengine/longview.cgi?view-av,554286&text=varicella>, 1 page. -- and

UNITED STATES PATENT AND TRADEMARK OFFICE

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PATENT NO. : 6,843,997 B2
APPLICATION NO. : 10/288823
DATED : January 18, 2005
INVENTOR(S) : Charles F. Grose et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

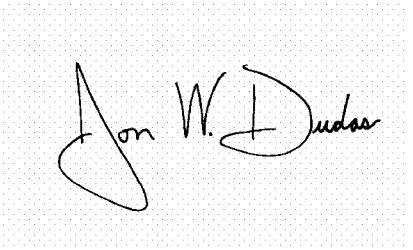
On The Title Page Item (56) under Other Publications (second column), delete "ATCC VR-1367, "Varicella-Zoster," [online]. Retrieved on June 5, 2001. Retrieved from the Internet: <URL:http://phage/atcc/org/cgi-bin/searchengine/longview.cgi!view=av, 871705, VR1367&text= VR-1367>, 2 pages." and insert -- ATCC VR-1367, "Varicella-Zoster," [online]. Retrieved on June 5, 2001. Retrieved from the Internet: <URL:http://phage/atcc/org/cgi-bin/searchengine/longview.cgi?view=av, 871705, VR-1367&text= VR-1367>, 2 pages. --; and

On The Title Page, Item (56) Pg 2 under Other Publications (first column), delete "P. LaRussa et al., "Restriction fragment length polymorphism of polymerase chain reaction products from vaccine and wild-type varicella-zoster virus isolates," *J. Virol. 66*, 1016-1020 (1922)." and insert -- P. LaRussa et al., "Restriction fragment length polymorphism of polymerase chain reaction products from vaccine and wild-type varicella-zoster virus isolates," *J. Virol. 66*, 1016-1020 (1992). --; and

On The Title Page Pg 2 Item (56) Other Publications (second column), delete "National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Genbank X04370. Accession Number X04370 M14891 M16612, "The complete DNA sequence of varicella-zoster virus," [online]. J. Gen. Virol. 67(Pt 9) 1759-1816 (1986), [retrieved on May 29, 2001]. Retrieved from the Internet:<URL:http://www.ncbi.nlm.nih.gov/entrez/query.fgci! cmd=Retrieve&db=Nuceloti de&list uids=599.8=Genbank>, 39 pages." and insert -- National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Genbank X04370. Accession Number X04370 M14891 M16612, "The complete DNA sequence of varicella-zoster virus," [online]. J. Gen. Virol. 67(Pt 9) 1759-1816 (1986), [retrieved on May 29, 2001]. Retrieved from the Internet<URL:http://www.ncbi.nlm.nih.gov/entrez/query.fgci? cmd=Retrieve&db=Nucelot ide&list uids=599.8=Genbank>, 39 pages. --.

Signed and Sealed this

Twentieth Day of November, 2007



JON W. DUDAS
Director of the United States Patent and Trademark Office