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The Varicella-Zoster Virus Genome

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Abstract

The varicella-zoster virus (VZV) genome contains at least 70 genes, and all but 6 have homologs in herpes simplex virus. Cosmids and BACs corresponding to the VZV parental Oka and vaccine Oka viruses have been used to "knock-out" 34 VZV genes. Seven VZV genes (ORF4, 5, 9, 21, 29, 62, and 68) have been shown to be required for growth in vitro. Recombinant viruses expressing several markers (e.g. beta-galactosidase, green fluorescence protein, luciferase) and several foreign viral genes (from herpes simplex, Epstein-Barr virus, hepatitis B, mumps, HIV and simian immunodeficiency virus) have been constructed. Further studies of the VZV genome, using recombinant viruses, may facilitate the development of safer and more effective VZV vaccines. Furthermore, VZV might be useful as a vaccine vector to immunize against both VZV and other viruses.

1 Genome Structure and Organization

Varicella-zoster virus (VZV) is an alphaherpesvirus that is in the same subfamily as herpes simplex virus (HSV) 1 and 2. VZV is a member of varicellovirus genus, along with equine herpesvirus 1 and 4, pseudorabies virus, and bovine herpesvirus 1 and 5. Ceropithecine herpesvirus 9 (simian varicella virus) is virus most homologous to VZV.

1.1 VZV genome

The complete sequence of the VZV genome was determined by Davison and Scott (1986). The prototype strain, VZV Dumas is 124,884 base pairs in length. The genome consists of a unique long region (UL) bounded by terminal long (TRL) and internal long (IRL) repeats, and a unique short region (US) bounded by internal short (IRS), and terminal short (TRS) repeats (Figure 1). The US region can orientate either of two directions, while the UL region rarely changes its orientation; thus, there are usually two isomers of the genome in infected cells. The genome is linear in virions with an unpaired nucleotide at each end. In VZV-infected cells the ends pair and the genome circularizes.

The genome has five repeat regions. Repeat region 1 (R1) is located in open reading frame (ORF) 11, R2 is located in ORF14 (glycoprotein C), R3 in ORF22, R4 between ORF62 and the origin of viral replication, and R5 between ORF 60 and 61. The length of the repeat regions varies among different VZV strains and has been used to distinguish the strains.

1.2 VZV genes

1.2.1 VZV immediate-early genes—VZV encodes at least 70 genes, three (ORF62, 63, 64) are which are present in both of the short repeat regions (Cohen et al. 2007b). VZV encodes at least 3 immediate-early (IE) proteins that are located in the tegument of virions and regulate virus transcription (Table 1). IE4 and IE62 transactivate IE, late, and early

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promoters. IE63 represses several VZV promoters, and inhibits the activity of interferon-alpha (Ambagala et al. 2007), and binds to anti-silencing protein 1 (Ambagala et al. 2009). ORF61 protein, which is not present in the tegument of virions and has not been shown to be an IE gene, activates IE, early, and late viral promoters.

1.2.2 VZV genes encoding replication proteins—VZV encodes a viral DNA polymerase, likely composed of two subunits (ORF28 and ORF16) that is inhibited by acyclovir. The viral thymidine kinase (ORF36) phosphorylates deoxycytidine, thymidine, and acyclovir. VZV ORF18 and ORF19 encode the small and large subunits of ribonucleotide reductase which convert ribonucleotides to deoxyribonucleotides. VZV encodes at least two DNA binding proteins- ORF29 protein is a single-stranded DNA binding protein, and ORF 51 protein binds to the origin of DNA replication. VZV encodes two protein kinases. ORF47 protein phosphorylates VZV ORF32 protein, IE62, IE63, and glycoprotein I. ORF66 protein phosphorylates IE62 which results inclusion of IE62 into the virion tegument. VZV encodes other enzymes including a dUTPase (ORF8), thymidylate synthetase (ORF13), protease (ORF33), DNase (ORF48), and uracil DNA glycosylase (ORF59).

1.2.3 VZV genes encoding putative late proteins—VZV ORF10 encodes a tegument protein that forms a complex with transcription factors at the ORF62 promoter to activate transcription of ORF62. ORF17 protein induces cleavage of RNA. ORF33.5 encodes the assembly protein which forms a scaffold thought be involved in construction of nucleocapsids. ORF40 encodes the major nucleocapsid protein, while ORF21 also encodes a nucleocapsid protein. ORF54 encodes the putative portal protein which allows viral DNA to enter nucleocapsids.

1.2.4 VZV genes encoding glycoproteins—VZV encodes 7 viral glycoproteins- gB (ORF31), gC (ORF14), gE (OEF68), gH (ORF 37), gI (ORF67), gK (ORF 5), gL (ORF 60), gM (ORF50), and presumably gN (ORF9A). VZV gB, based on homology with HSV gB, is likely critical for entry of virus into cells. gE is binds to a cellular receptor (insulin degrading enzyme [Li et al. 2006]) and gH and gM are important for cell-to-cell spread of virus (Yamagishi et al. 2008). gI facilitates maturation of gE ,and gL is a chaperone for gH. gK may be important for syncytia formation.

2 Comparative genomics of VZV and HSV

VZV and HSV are largely collinear, although the UL region of HSV is orientated in the opposite direction to that of VZV using the standard nomenclature. The VZV US region is much shorter (5.2 kb) than HSV-1 US (13.0 kb) and the VZV TRL and IRL regions are also shorter (0.9 kb) than their HSV-1 counterparts (9.2 kb).

2.1 Core proteins conserved with herpesviruses in other subfamilies

The VZV genome contains about 41 “core genes” that are conserved with each of the three subfamilies of herpesviruses, alphaherpesvirus, betaherpesvirus, and gammaherpesvirus (Davison 1993). Core genes include IE4, the VZV DNA polymerase, helicase-primase components, single-stranded DNA-binding protein, ribonucleotide reductase, uracil-DNA glycosylase, dUTPase, DNase, ORF47 protein kinase, major capsid protein, protease, assembly protein, several tegument proteins, gB, gH, gL, gM, and gN.

2.2 VZV functional and nonfunctional homologs of HSV genes

Several VZV genes can complement their HSV homologs. VZV ORF61 can substitute for HSV ICP0 (Moriuchi et al. 1992) and VZV ORF62 can complement HSV ICP0 (Felser et al.

1988). Although VZV ORF10 can complement the transactivating function of HSV VP16, HSV-1 VP16 is essential for replication of HSV while VZV ORF10 is dispensable (Cohen and Seidel 1994b; Moriuchi et al. 1993). VZV ORF 51 can complement HSV UL9 (Chen et al 1995). In contrast, VZV ORF4 cannot complement HSV ICP27 (Moriuchi et al 1994a).

2.3 VZV genes not conserved with HSV

VZV encodes 6 genes (ORF1,2,13,32,57, and S/L) that are absent in HSV (Figure 1). ORF 13 encodes the viral thymidylate synthetase which has a homolog in herpesvirus saimiri and Kaposi's sarcoma associated herpesvirus.

2.4 HSV genes not conserved with VZV

HSV encodes 9 genes (UL45, UL56, US2, US5, US6, US11, US12, and LAT) that are absent in VZV (Figure 1). HSV US 12 encodes ICP47, which blocks presentation of MHC class I. HSV US6, US 4, and US5 encode glycoproteins D, G, and J, respectively. VZV gE is the most abundant viral glycoprotein and shares many features of HSV gD, including binding to a cellular receptor which contributes to VZV entry (Li et al. 2007).

3 Mutagenesis with cosmids and BACs

3.1 Mutagenesis using marker rescue

The first genetically engineered mutant of VZV was constructed in 1987 in which the Epstein-Barr virus gp350 gene was inserted into the VZV genome (Lowe et al. 1987). Fibroblasts were cotransfected with VZV viron DNA and a plasmid with EBV gp350 flanked by VZV thymidine kinase sequences and plaques were purified by limiting dilution. While the procedure was successful, the process of plaque purification which requires sequential rounds of sonication is very labor intensive. The same strategy in which VZV virion DNA is contransfected with plasmids with flanking sequences that are homologous to VZV DNA was used to reinsert and thereby "rescue" essential genes into the genome of VZV lacking ORF4 (Cohen et al.. 2005), ORF21 (Xia et al. 2003), and ORF68 (Ali et al. 2009).

3.2 Mutagenesis using cosmids

A cosmid system was first used to generate mutations in the VZV genome in 1993 (Cohen and Seidel 1993). Virion DNA was isolated from the Oka vaccine strain of VZV, the linear DNA was blunted with T4 DNA polymerase, and oligonucleotides containing Not I or Mst II restriction sites were ligated to the DNA. The resulting DNA was cut with Mst II or Not I and four large DNA fragments, which overlap the entire VZV genome were ligated into a cosmid vector that was linearized with Mst II or Not I. Human melanoma cells were transfected with the four cosmids along with a plasmid encoding VZV IE62 which increases the infectivity of viral DNA (Moriuchi et al 1994b). While the cosmids are able to produce infectious virus in the absence of the plasmid, the latter increases the reliability and enhances the efficiency of virus production. Recombinant VZV derived from the cosmids grew to similar titers as the nonrecombinant virus used to generate the cosmids.

Other cosmid systems have also developed to perform VZV mutagenesis. Mallory et al. (1997) used 5 cosmids derived from the Oka vaccine strain to produce recombinant virus and Niizuma et al. (2003) described a cosmid library from the parental Oka vaccine. These cosmids have been used to "knock-out" a large number of VZV genes and to study the phenotype of the resulting mutants in cell culture and rodents.

3.3 Mutagenesis using BACs

Four separate BACs have been constructed for VZV mutagenesis. Three research groups have inserted the parental Oka virus into BACs (Nagaike et al. 2004; Zhang et al. 2007; Tischer et al. 2007) and one group inserted the vaccine Oka virus into a BAC (Yoshii et al. 2007). While each of the recombinant parental Oka viruses derived from BACs grew to titers similar to nonrecombinant parental Oka virus, recombinant virus derived from the vaccine Oka BAC grew to slightly lower titers than the nonrecombinant vaccine Oka virus.

3.4 Results of mutagenesis studies

VZV cosmids and BACs have been used to mutate multiple VZV genes (Table 2). Several VZV genes (ORF4, 5, 9, 21, 29, 62, and 68) have been shown to be essential for replication in cells in vitro. Other VZV genes that have been tested are not required for growth in cell culture, but some are required for growth in certain types of cells in vitro (Cohen and Nguygen 1997), in lymphocytes (Moffat et al. 1998; Song et al. 2000), or in human skin (Moffat et al. 1998, and Chapter 12). Each of the 6 VZV genes which do not have HSV homologs (ORF1, 2, 13, 32, 57, and S/L) are not required for growth in cell culture (Cohen and Seidel 1995; Cohen and Seidel 1993; Cox et al. 1998; Reddy et al. 1998a; Sato et al. 2002b; Zhang et al. 2007).

Chimeras have been constructed containing various portions of the parental and vaccine Oka genomes (Zerboni et al. 2005). These chimeras have demonstrated that attenuation of VZV is a multigenic trait due to mutations throughout the genome.

VZV cosmids has been used to prove that mutations in individual genes correspond with resistance to antiviral compounds. Mutagenesis of VZV ORF54 (homologous to the portal protein of HSV) conferred resistance to a thioruea inhibitor compound (Visalli et al. 2003). VZV cosmids have been used to produce virus expressing beta-galactosidase (Cohen et al. 1998), green fluorescence protein (Zerboni et al. 2000, Li et al. 2006), or luciferase (Oliver et al. 2008, Zhang et al. 2007) which have been useful for studies in animals and virus entry in vitro.

Most recombinant viruses have stable genomes with a few exceptions. Attempts to delete or mutate one copy of a gene that is normally present in both of the short repeat regions of the genome often result in recombination with wild-type sequences in both short repeat regions after several rounds of replication (Sommer et al. 2001; Sato et al. 2003a; Oliver et al. 2008). In addition, point mutations that severely impair growth of the virus can sometimes undergo back mutation with reversion to wild-type virus over time (Cohen et al. 1998)

VZV genes thought to be required for cell growth have been proven to essential by growing a virus mutant unable to express the protein in a complementing cell line (Xia et al. 2003) or in cells infected with baculovirus expressing the VZV protein (Ali et al. 2009; Cohen et al. 2005; Cohen et al 2007) and then showing that the virus cannot be grown on non-complementing cells. An alternative approach has been to insert the essential gene elsewhere in the viral genome. This latter method is not ideal, since the gene will not be formally proven to be required for growth. Inefficiency in cosmid transfections or impaired virus growth may be misinterpreted as showing that a virus gene is essential. One VZV gene, ORF63, has been reported to be essential in the absence of a complementation system (Sommer et al 2001), but was actually shown to be non-essential for growth in cell culture (Cohen et al. 2004). The failure to obtain virus in the former study may have been due to the lack of a cotransfected plasmid expressing the VZV IE62 gene to transiently enhance virus growth after cosmid DNA transfection (Baiker et al. 2004).

3.5 VZV as an expression vector

VZV has been used to express a number of foreign viral proteins (Table 3). While these recombinant VZV mutants have been shown to induce immunity to the foreign virus and to protect animals from disease after challenging with the foreign virus in some cases (Heineman et al. 1995; Heineman et al. 2004), in one case the recombinant VZV actually enhanced replication of the foreign virus (Strapans et al. 2004). Thus, it is critical to test such recombinant viruses in animal models whenever possible. Since children are vaccinated with the varicella vaccine to prevent chickenpox, expression of additional viral proteins might allow immunization against other viruses and ultimately reduce the number of vaccines required during childhood.

3.6 Use of genetics to develop safer VZV vaccines

The current vaccine is very safe in immunocompetant persons, but it has caused disease in severely immunocompromised persons. Replication-defective vaccines might be developed by growing viruses in complementing cells; however, attempts to produce high titers of cell-free virus using this approach has been unsuccessful (Cohen, unpublished data). An alternative approach is to express an essential gene, such as one expressed during latency, under a different promoter, which may allow high titers of virus during replication, but may impair latency (Cohen et al 2007).

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Abbreviations

VZV	varicella-zoster virus
HSV	herpes simplex virus
UL	unique long
TRL	terminal repeat long
IRL	internal repeat long
US	unique short
IRS	internal repeat short
TRS	terminal repeat short
IE	immediate-early

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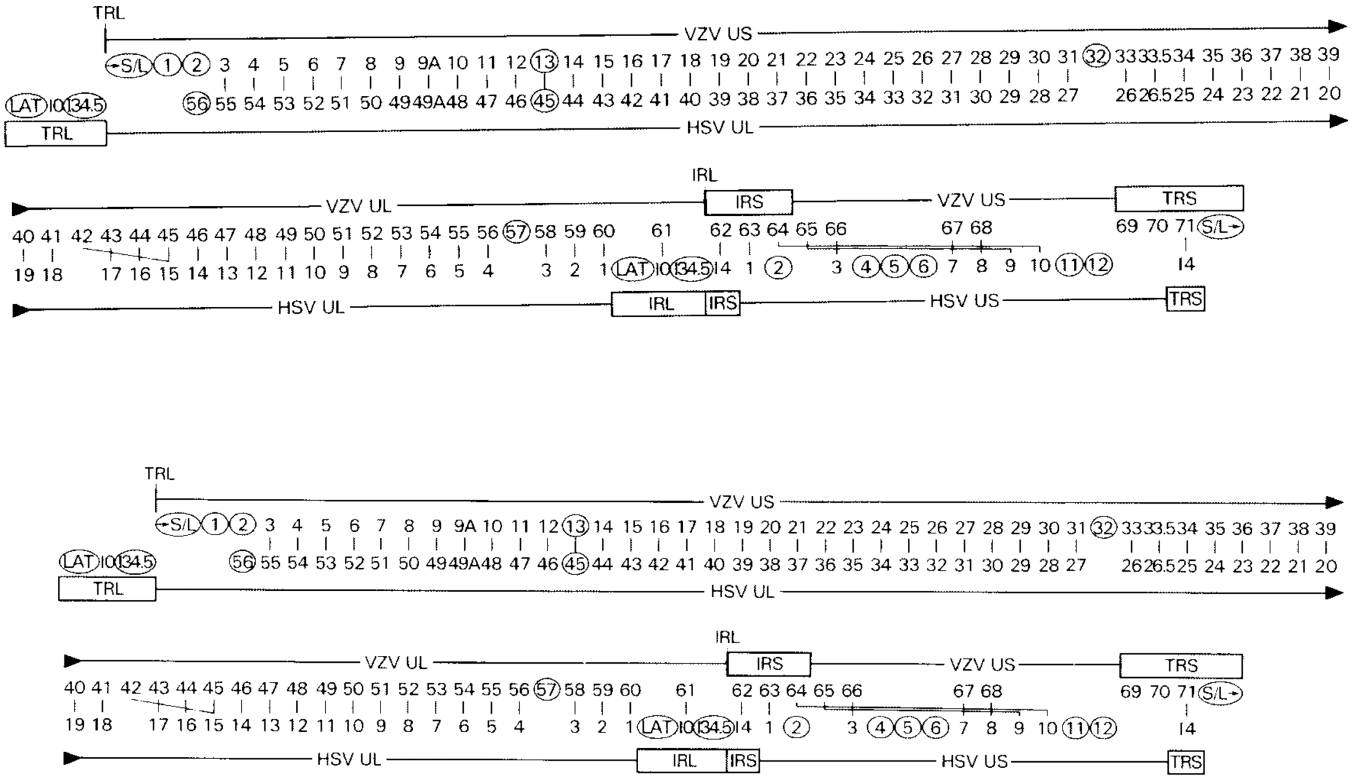

Figure 1.

Comparison of the VZV and HSV genomes. The VZV genome (first rows) contains unique long (UL) and unique short (US) regions flanked by terminal long (TRL), terminal short (TRS), internal long (IRL), and internal short (IRS) repeats. VZV genes (second rows) and HSV genes (third rows) are shown by numbers. VZV or HSV genes that do not have homologs in the corresponding virus circled. The HSV genome contains UL and US regions, and TRL, TRS, IRL, and IRS repeats (fourth rows). Modified from Cohen 1999, with permission.

Table 1

VZV gene products

VZV gene	HSV-1 homolog	VZV protein function
1	None	Membrane protein
2	None	
3	UL55	
4	UL54 (ICP 27)	Transactivator, tegument protein, expressed in latency
5	UL53 (gK)	gK
6	UL52 (HPC)	
7	UL51	
8	UL50	Deoxyuridine triphosphatase
9	UL49 (VP22)	Tegument protein
9A	UL49A	Syncytia formation, putative gN
10	UL48 (VP16)	Transactivator, tegument protein
11	UL47 (VP13/14)	
12	UL46 (VP11/12)	
13	None	Thymidylate synthetase
14	UL44 (gC)	gC
15	UL43	
16	UL42 (PPF)	Putative small subunit of viral DNA polymerase
17	UL41 (vhs)	Induces RNA cleavage
18	UL40	Ribonucleotide reductase, small subunit
19	UL39	Ribonucleotide reductase, large subunit
20	UL38 (VP19C)	
21	UL37	Nucleocapsid protein, expressed in latency
22	UL36	
23	UL35 (VP26)	Capsid assembly
24	UL34	
25	UL33	
26	UL32	
27	UL31	
28	UL30	DNA polymerase
29	UL29 (ICP8)	ssDNA binding protein, expressed in latency
30	UL28	
31	UL27 (gB)	gB
32	None	Probable substrate for ORF47 kinase
33	UL26 (VP24)	Protease
33.5	UL26.5 (VP22)	Assembly protein
34	UL25	
35	UL24	Cell-to-cell fusion
36	UL23	Thymidine kinase
37	UL22 (gH)	gH

VZV gene	HSV-1 homolog	VZV protein function
38	UL21	
39	UL20	
40	UL19 (VP5)	Major nucleocapsid protein
41	UL18 (VP23)	
42/45	UL15	
43	UL17	
44	UL16	
46	UL14	
47	UL13	Protein kinase, tegument protein
48	UL12	Putative DNase
49	UL11	Virion protein
50	UL10 (gM)	gM
51	UL9	Origin binding protein
52	UL8 (HPC)	
53	UL7	
54	UL6	Putative portal protein
55	UL5 (HPC)	
56	UL4	
57	None	Cytoplasmic protein
58	UL3	
59	UL2	Uracil-DNA glycosylase
60	UL1 (gL)	gL, chaperone for gH
61	ICP0	Transactivator, transrepressor
62,71	ICP4	Transactivator, tegument protein, expressed in latency
63,70	US1 (ICP22)	Tegument protein, transrepressor, inhibits interferon- alpha, expressed in latency
64,69	US10	
65	US9	Virion protein
66	US3	Protein kinase, expressed in latency
67	US7 (gI)	gI
68	US8 (gE)	gE
S/L	None	Cytoplasmic protein (also referred to as ORF0)

HPC=helicase-primase complex, PPF=polymerase processivity factor

Table 2

Deletion and stop codon mutants constructed using cosmids or BACs in VZV and their effect on growth in vitro.

VZV Gene	Mutation	Growth in Vitro	Reference
ORFS/L (ORF0)	del	impaired	Zhang et al. 2007
ORF1	stop	no change	Cohen and Seidel 1995
	del	slight reduced	Zhang et al. 2007
ORF2	del	no change	Sato et al. 2002b
	del	no change	Zhang et al. 2007
ORF3	del	slight reduced	Zhang et al. 2007
ORF4	del	essential	Cohen et al. 2005
	del	essential	Sato et al. 2003b
	del	essential	Zhang et al. 2007
ORF5 (gK)	del	essential	Mo et al. 1999
ORF8	stop	no change	Ross et al. 2007
	delete +	reduced	Ross et al. 2007
ORF9	start	essential	Tischer et al. 2007
	del	essential	Che et al. 2008
ORF9A	stop	no change	Ross et al. 1997
ORF10	del	no change	Cohen and Seidel 1994b
	del	no change	Che et al. 2008
ORF11	del	no change	Che et al. 2008
ORF12	del	no change	Che et al. 2008
ORF13	stop	no change	Cohen and Seidel 1993
ORF14 (gC)	stop	no change	Cohen and Seidel 1994a
ORF17	del	reduced	Sato et al. 2002a
ORF19	del	reduced	Heineman and Cohen 1994
ORF21	del	essential	Xia et al. 2003
ORF23	del	reduced	Chaudhuri et al. 2008
ORF29	del	essential	Cohen et al. 2007
ORF32	del	no change	Reddy et al. 1998a
ORF35	del	reduced	Ito et al. 2005
ORF47	stop	no change	Heineman and Cohen 1995
ORF49	del	reduced	Sadaoka et al. 2007
ORF50	del	reduced	Yamagishi et al. 2008
ORF57	del	no change	Cox et al. 1998
ORF58	del	no change	Yoshii et al. 2008
ORF59	del	no change	Reddy et al. 1998b
ORF61	del	reduced	Cohen and Nguyen 1998
ORF62	del	essential	Sato et al. 2003a
ORF63	del	essential	Sommer et al. 2001
	del	reduced	Cohen et al. 2004

VZV Gene	Mutation	Growth in Vitro	Reference
ORF65	del	no change	Cohen et al. 2001
	del	no change	Niizuma et al. 2003
ORF66	stop	no change	Heineman et al. 1996
ORF67 (gI)	del	reduced * or essential	Cohen and Nguyen 1997
	del	reduced	Mallory et al. 1997
ORF68 (gE)	del	essential	Mallory et al. 1997
	del	essential	Ali et al. 2009

+ interrupts expression of ORF8 and ORF9A

* reduced in melanoma cells, essential for Vero cells

Stop=stop codons, del=deletion, start=mutations in start codon

Table 3

Recombinant VZV expressing other viral proteins

Viral protein	Immunogenicity	Reference
EBV gp350	Not reported	Lowe et al. l987
Hepatitis B SAg	Induced antibody to Hepatitis B S Ag	Shiraki et al. 1991
HIV env	Induced humoral and cellular immunity to HIV	Shiraki et al. 2001
SIV gp160	Induced neutralizing antibody Enhanced SIV infection in monkeys	Strapans et al. 2004
HSV gD	Induced neutralizing antibody to HSV-2 Reduced severity of HSV-2 in guinea pigs	Heineman et al. 1995
Mumps HA-N	Induced neutralizing antibody to mumps	Somboonthum et al. 2007
HSV gD and gB	Induced neutralizing antibody to HSV-2 Reduced severity of HSV-2 in guinea pigs	Heineman et al. 2004

gp350=glycoprotein 350, SAg=surface antigen, gD=glycoprotein Dm HA-N=hemagglutinin-neuraminidase, gB=glycoprotein B