

Varicella Zoster Virus: Out of Africa and into the Research Laboratory

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KEY WORDS

z VARICELLA ZOSTER VIRUS z HERPES ZOSTER z VIRAL EVOLUTION
z VIRAL PATHOGENESIS z VIRAL REPLICATION z GENOMICS z SINGLE
NUCLEOTIDE POLYMORPHISMS z MUTANT VIRUSES

SUMMARY

This review updates on numerous topics relating to the evolutionary origins of varicella zoster virus (VZV), the replication cycle, virion assembly, and the recent genomic analyses. VZV is one of eight human herpesviruses that have existed for at least 400 million years. It has co-evolved with humankind and is present in all nationalities globally. The pathogenesis of varicella (chickenpox) is dependent on viral replication and dispersion through the body in T-lymphocytes. VZV replication is similar to that of herpes simplex virus. A complete analysis of VZV transcripts has identified their relative abundance, with the transcripts for the regulatory proteins (open reading frame) ORF62 and ORF63 among the greatest. Studies of virion assembly have shown that endocytosis pathways are involved in the envelopment process by the viral glycoproteins. The complete sequencing of five VZV strains has identified numerous single nucleotide polymorphisms, and in turn, VZV strains have been segregated into European/North American and Asian clades. Furthermore, a small number of mutant VZV strains have been identified. These results suggest more diversity between VZV strains than previously recognized.

Introduction

VARICELLA ZOSTER VIRUS (VZV) has received considerable attention over the last decade because it is the first human herpesvirus for which a vaccine has been licensed for prevention of varicella (chickenpox). The vaccine has now been administered to millions of children around the world,¹ and extensive reviews of it have been recently published.²⁻⁴ The present review provides an update on a wide range of topics relating to the evolutionary origins of VZV, the replication cycle and virion assembly and, finally, the most recent genomic analyses, including the documentation of North American/European and Asian clades. It demonstrates that VZV is a virus that has co-evolved successfully with humankind in a more complicated pattern than other viruses commonly associated with childhood illnesses, such as measles and rubella. Articles have been selected to illustrate specific points and therefore the reference list is not intended to be comprehensive.

Origins of Varicella Zoster Virus in Africa

Varicella zoster virus is an ancient virus. The viral genome consists of slightly fewer than 125 000 bases,⁵ and as such, VZV has the smallest genome of the eight



Figure 1:
Prosimians in Africa. Primates such as the angwantibo living currently in Africa closely resemble ancestral primates. The mother primate in this drawing 'baby-parks' her infant between her hind legs, while foraging for food. Early primates evolved at the same time as varicella zoster virus first appeared about 50 – 70 million years ago. Reproduced with permission from Princeton University Press.

human herpesviruses. In one sense, therefore, VZV is a minimalist version of a human herpesvirus. On a geological time scale, herpesviruses have existed since the Devonian period, 400 million years before the present time,⁶ when living creatures included sharks, early bony fish and early amphibians. The most primitive known herpesvirus exists today in oysters.⁷ Molecular phylogenetic analyses indicate that the origins of the ancestral VZV date from about 70 to 100 million years ago, during the Cretaceous period. Living creatures of that era were dinosaurs, reptiles, amphibians, snakes, early birds and early mammals. Thereafter, the human herpesviruses, including VZV, co-evolved in ancestral primates that were derived from small mammals, between 50 and 70 million years ago (Figure 1).

During the subsequent major branching of simians and great apes, ancestral VZV co-speciated with the apes and eventually with hominids, as they evolved in Africa 6 million years ago. The well-known Lucy (*Australopithecus*) would have carried VZV over

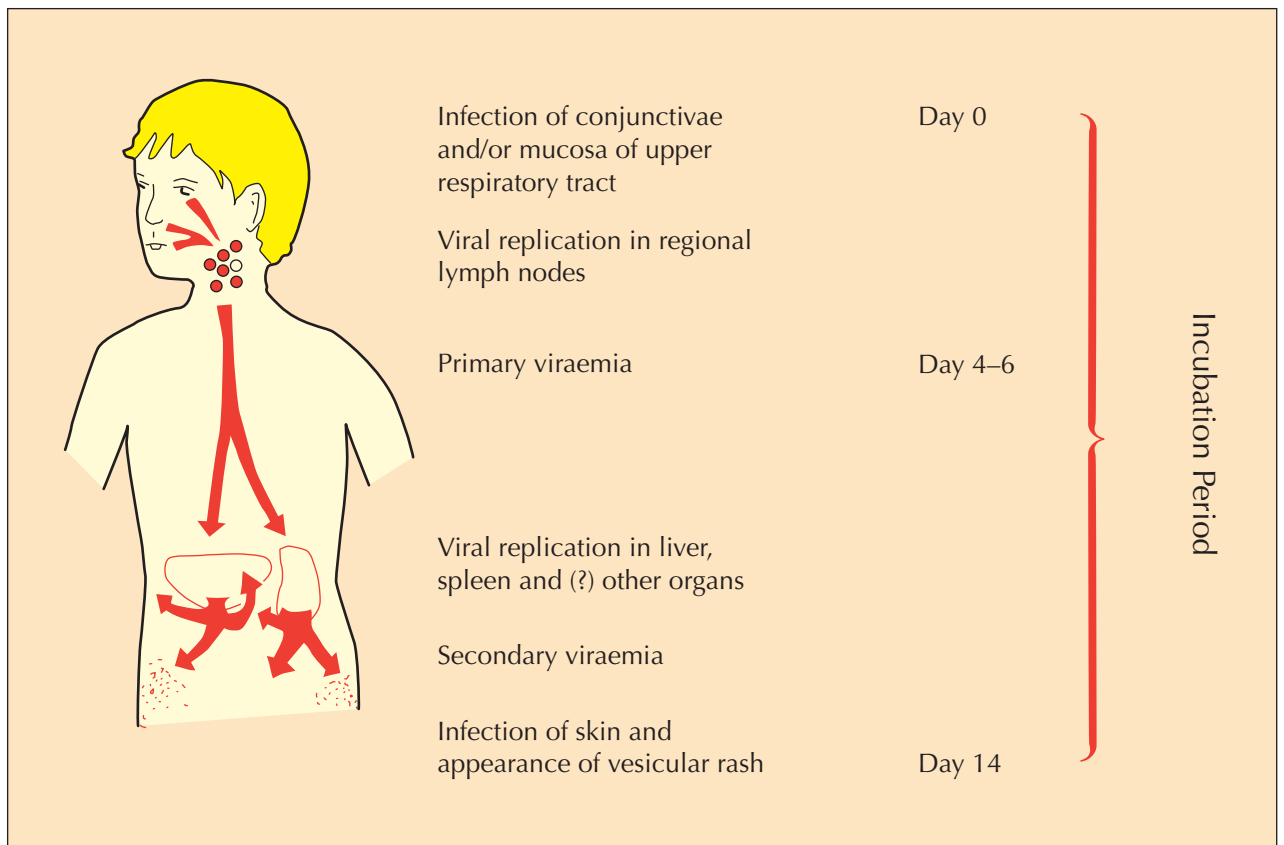


Figure 2: Model for the pathogenesis of varicella zoster virus infection. This model includes two phases of infection within the incubation period of two weeks. Reproduced with permission from the American Academy of Pediatrics.

3 million years ago in East Africa. Similarly, *Homo erectus* and finally *Homo sapiens* carried VZV. An increase in the population of the highly adapted *Homo sapiens* in Africa was followed by migrations out of Africa, initially to India and China and subsequently to Europe. *Homo sapiens* arrived in North America from Asia via the Bering land bridge at least 10 000 to 15 000 years ago.

Zoster as a Survival Strategy

Varicella is found even in the most primitive and isolated populations. These population studies provide important insights into the persistence of this viral disease throughout the millennia. One investigation of particular interest included the small tribes on the periphery of the Amazon basin in Brazil, who had had little or no contact with neighbouring peoples when they were first found in the 1950s.⁸ When members of some of these tribes were tested for antibodies to different viruses, they were found to have serological evidence of past VZV infection. Varicella was not unusually virulent in the tribal members, nor did the disease threaten the continued existence of the tribe. The above studies document that varicella has been successfully transmitted from generation to generation since the arrival of humankind in the Americas over 10 000 years ago.

The mode of VZV transmission in very isolated populations is also illustrated by examples from the Shetland Islands, off the Scottish coast, in the early 1900s.⁹ On some of these remote islands, the inhabitants lived on their own small enclosed farmsteads, widely separated from one another. Varicella in the children only followed a case of shingles in an adult, who was often a school teacher. Thus, the usual mode of transmission in primitive or isolated societies depended

upon reactivation of the virus as zoster in adults and its subsequent transmission to children as varicella. Alone among the human herpesviruses, VZV has this unusual survival strategy.

Pathogenesis and Animal Models

A widely cited model for varicella is based on the schema for the pathogenesis of mousepox.⁹ VZV infection is acquired by small droplets containing live virus, which fall upon the conjunctivae and upper respiratory tract of the index case (Figure 2). The virus invades and enters the regional cervical lymph tissue, where local replication occurs. Another possible site of primary replication is the tonsillar tissue. After a 4–6-day period, a sufficient quantity of infectious virus is released to cause a primary viraemia.

During the primary viraemia, the virus is disseminated throughout the body in T-lymphocytes.¹⁰ Known secondary sites of viral replication include epithelial derivatives, such as the columnar epithelium of the respiratory tree, as well as the endothelial lining of blood vessels. Between 4–6 days after the primary viraemia, the secondary viraemia takes place. At this time, the virus exits the capillaries and travels to the epidermis, forming the characteristic varicella vesicle.

The above schema is currently being modified, based on recent information gained from experiments carried out in the severe combined immunodeficient (SCID) mouse model.¹¹ In SCID mouse experiments, VZV tropism for human T-cells was demonstrated by infecting human thymus and liver xenografts.¹² VZV was subsequently located within CD4 and CD8 T-cells but not in B-cells. In the human, the tonsils contain many T-cells and VZV preferentially infects activated memory CD4 cells. T-cell populations that expressed the skin homing markers for cutaneous leucocyte antigen

(CLA) and chemokine receptor 4 (CCR4) were also preferentially infected. In subsequent experiments, VZV-infected tonsillar T-cells were given, by intravenous infusion, to SCID mice with human skin xenografts. Within 10–21 days, infectious virus was recovered from the xenografts, demonstrating that the human skin implant had been infected by VZV carried within the T-cells circulating in the bloodstream of the SCID mouse. Furthermore, the extent of replication within the skin was modulated by the innate epidermal cell responses.¹³ Future studies will certainly elucidate the relevance of components of the different pathogenesis schemes.

VZV Replication and Virion Assembly

In cell culture, the titre of VZV is extremely low, in part because of a dependence on the mannose-6-phosphate receptor.¹⁴ In turn, the regulatory mechanisms affecting VZV replication resemble those already described for HSV replication. Based on an operational definition, there are a minimum of three sequential phases for the transcription of viral DNA: these phases correspond to the synthesis of three groups of polypeptides, which are designated immediate early (IE or α), early (β) and late (γ) proteins. Altogether, the VZV genome contains about 70 open reading frames (ORFs) within its 125 000 bases. Many of the protein products remain to be characterized. The most important VZV regulatory protein is called IE62 (ORF 62), the homologue of herpes simplex virus (HSV) ICP4.¹⁵ IE62 appears to be critical during lytic infection,¹⁶ whereas a second regulatory protein called IE63 (ORF63) is critical during establishment of latency.¹⁷ Recently, an extensive analysis of the relative abundance of the viral transcripts has been completed.¹⁸ Table 1 includes a listing of the transcripts encoding many of the important ORFs that have been investigated to date.

Table 1: Relative abundance of varicella zoster virus transcripts. Open reading frame 9 (ORF 9) is the most abundant transcript and ORF 40 is the least abundant

ORF	Abundance	Function
9	1	tegument
33	3	protease
63	5	regulatory
62	7	regulatory
1	13	membrane
10	17	regulatory
61	18	regulatory
68	19	glycoprotein E
67	22	glycoprotein I
28	24	polymerase
37	25	glycoprotein H
29	29	DNA binding
4	30	regulatory
60	41	glycoprotein L
31	43	glycoprotein B
40	61	major capsid

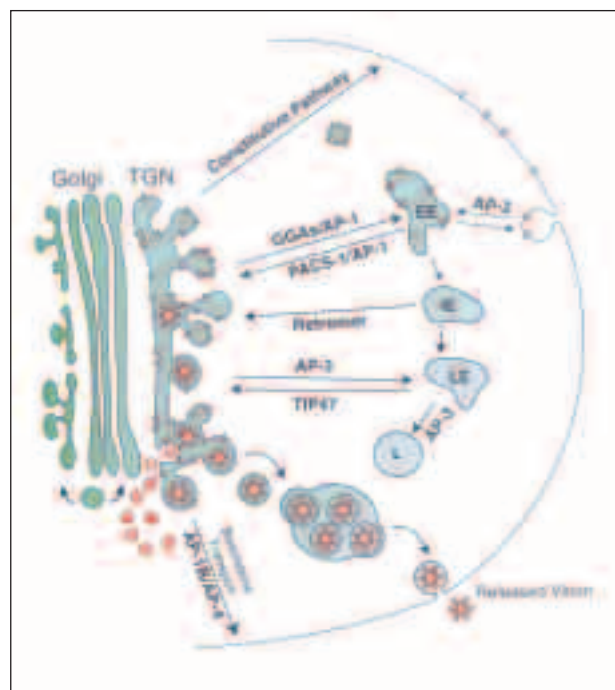


Figure 3: Trafficking pathways of varicella zoster virus (VZV) glycoproteins in the infected cell. The viral glycoproteins (red triangles) may follow several late secretory trafficking pathways to the TGN. Retrograde transport from the early endosomes to the TGN may occur via PACS-1 and AP-1. In addition, transport through sorting pathways from IE via the retromer complex or from late endosomes LE via TIP47 protein may also occur. Anterograde transport from the TGN to early endosomes is mediated by GGA and AP-1 proteins or to late endosomes by AP-3 protein. The VZV capsids (red hexagons) bud into the TGN-derived vacuoles, forming the viral envelope of membranes enriched with viral glycoproteins. The virion-laden vacuoles subsequently fuse with the outer cell membrane to release the infectious virions onto cell surface. Data from Grose et al.¹⁹ TGN, trans-Golgi network; EE, early endosomes; PACS, phosphorin acidic cluster sorting protein; AP, adaptor protein; IE, intermediate endosomes; LE, late endosomes; TIP47, tail-interacting protein of 47kDa; GGA, Golgi-localized, gamma ear-containing ADP ribosylation factor-binding protein; L, lysosome.

The events which occur during virion assembly remain a subject of diverse opinion. One model proposes that progeny nucleocapsids acquire their initial envelope at the inner nuclear membrane. De-envelopment of the nucleocapsid appears to take place as the capsid exits the outer nuclear membrane and enters the cytoplasm. Within the cytoplasm, re-envelopment may occur as the nucleocapsids enter cytoplasmic vacuoles, presumably derived from the trans-Golgi network or a late endosome. The mechanisms by which the viral glycoproteins travel to the cytoplasmic vacuoles have been a subject of great interest (Figure 3). One possibility involves the endocytosis pathways.¹⁹ Several of the VZV glycoproteins, gE, gI, gH and gB, harbour endocytosis signals in their cytoplasmic tails.²⁰ Thus, they travel to the outer cell membrane after biosynthesis in the infected cell, subsequently move into the cell by endocytosis and then traffic through defined cellular pathways to early and late endosomes.²¹ Finally, the glycoproteins cluster in vacuoles derived from the trans-Golgi network, where envelopment occurs (Figure 4). The cytoplasmic vacuoles containing

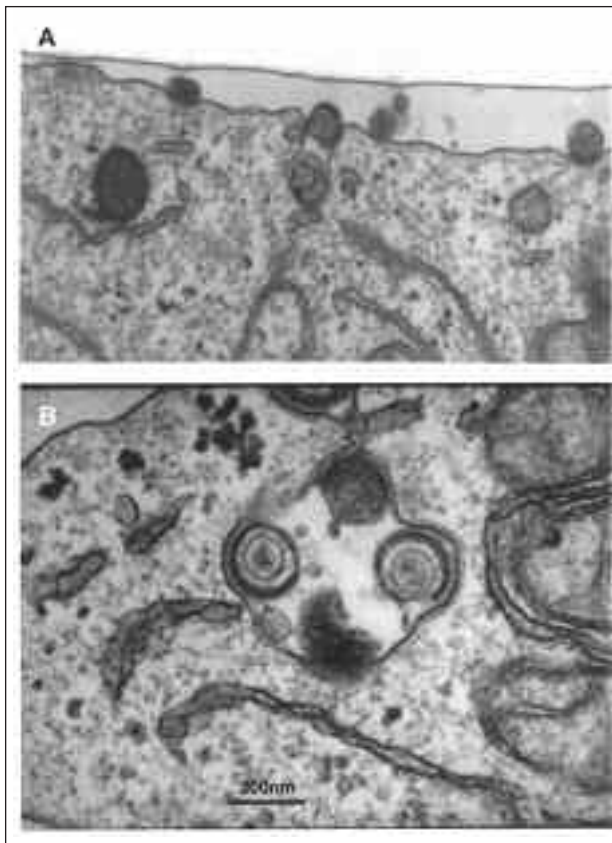


Figure 4:
Electron micrography of varicella zoster virus cytoplasmic envelopment. (A) Enveloped virions egress onto the surface of an infected cell. (B) Enveloped virions are present within a cytoplasmic vacuole prior to egress. These structures are compatible with the re-envelopment model of virion assembly, since these are the likely site of envelopment by the viral glycoproteins, such as gE.

enveloped virions travel to the outer membrane, where the enveloped viral particles are released by exocytosis. VZV particles emerge onto the cell surface in long rows called viral highways (Figure 5).

Genomics, Clades and Mutants

Currently, five complete VZV DNA sequences are present in GenBank (International Nucleotide Sequence Database Collaboration and GenBank National Center for Biotechnology, National Library of Medicine, Bethesda, USA). These include the original Dumas sequence published by Davison and Scott in 1986.⁵ Subsequently, the sequences of the Japanese Oka wild-type and vaccine strains were published.²² Most recently the sequences of the two VZV mutant viruses have been published.²³ When these sequences were compared, they were highly conserved (greater than 99%). Nevertheless, differences of less than 1% may be critical for differences in the biological properties of VZV strains. For example, the Oka vaccine strain is obviously attenuated and one of the most likely candidate genes for attenuation is VZV IE62.^{23–25} The IE62 protein contains several missense mutations. It is likely, however, that changes in IE62 alone do not cause attenuation, but they must be present in combination with small changes elsewhere in the genome.

In addition, several strains have been partially sequenced and single nucleotide polymorphisms have been identified. These include strains from Europe, North America and Asia.^{22,25–27} Phylogenetic analysis

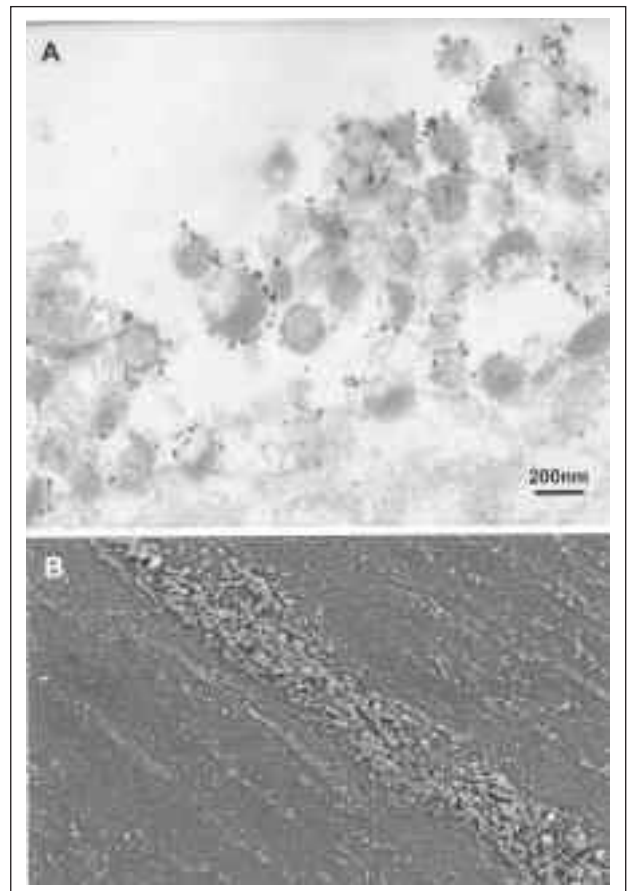


Figure 5:
Electron micrography of viral highways. (A) Virions at the infected cell surface are labelled with gold beads attached to anti-varicella zoster virus glycoprotein E monoclonal antibody. (B) Virions on the infected cell surface emerge in long pathways, sometimes called viral highways.

generally showed that the strains clustered broadly into two clades representing North America/Europe and Asia.²⁵ The Dumas strain is now accepted as the prototype of an European/North American strain, while Oka is the same for Asian strains. Thus VZV phylogeography does mimic the great migrations of humankind out of Africa.

Finally, contrary to all expectations, VZV mutant viruses have been discovered in the community. The first mutant to be discovered was called VZV-MSP, because it was found in Minneapolis/St Paul, USA.²⁸ The case involved a young child with leukaemia, who contracted varicella and was hospitalized. A routine viral culture grew a VZV strain that could not be typed because the site of monoclonal antibody attachment of the gE glycoprotein had been mutated. In other words, a B-cell epitope had been lost secondary to a missense mutation. A second similar mutant was isolated from a shingles patient in Vancouver, Canada, and recently, two additional VZV gE mutant viruses were documented by virologists at the Karolinska University Hospital in Stockholm, Sweden (Wirgart B, personal communication, 1 August 2005). In light of the fact that four viruses which mutated in the same gE epitope have now been discovered, the most likely explanation is immunological pressure rather than gradual evolutionary adaptation. The sequence of the original VZV-MSP mutant virus was patented, because of the unprecedented nature of the genetic change in the gE protein, which suggested a second VZV serotype.

Conclusions

The challenges for future VZV research are daunting. VZV is unlike many other childhood viruses because of its latent state and subsequent reactivation as shingles. Results based on a qualitative mathematical analysis of an epidemiological model of varicella and zoster have suggested that VZV would persist in populations as long as there were even a few individuals in that population who might develop shingles.⁸ In spite of vaccination, therefore, VZV is likely to recur for the next 70–90 years, even within a highly vaccinated population. Whether mutations will occur over that time period is unknown, but the identification of at least four mutant viruses in the 1990s indicates that the virus is capable of genetic change. Whether these mutations will ever alter the efficacy of varicella vaccination is an unanswerable question in 2006.

Further studies of VZV replication, including further analyses of the abundance of VZV transcripts and their protein products, will clarify the phased steps within the infectious cycle. Additional analyses of VZV trafficking pathways will clarify the sequential steps required for envelopment, de-envelopment and re-envelopment during the assembly of the virion. The above studies will eventually reveal the nature of this reclusive herpesvirus, which has so successfully

adapted itself to survive within human hosts living everywhere from Africa, to the Amazon basin, to the major metropolitan areas of the world.

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Conflicts of Interest

No conflicts of interest were declared in relation to this article.

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