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Molecular mechanisms of varicella zoster virus pathogenesis

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Abstract

Varicella zoster virus (VZV) is the causative agent of varicella (chickenpox) and zoster (shingles). Investigating VZV pathogenesis is challenging as VZV is a human-specific virus and infection does not occur, or is highly restricted, in other species. However, the use of human tissue xenografts in mice with severe combined immunodeficiency (SCID) enables the analysis of VZV infection in differentiated human cells in their typical tissue microenvironment. Xenografts of human skin, dorsal root ganglia or foetal thymus that contains T cells can be infected with mutant viruses or in the presence of inhibitors of viral or cellular functions to assess the molecular mechanisms of VZV–host interactions. In this Review, we discuss how these models have improved our understanding of VZV pathogenesis.

Varicella zoster virus (VZV), which is a human alphaherpesvirus of the genus *Varicellovirus*, causes varicella (also known as chickenpox) and zoster (also known as shingles)[1]. Epidemiological evidence suggests that primary VZV infection begins with replication in epithelial cells of the upper respiratory mucosa, which is followed by the widely distributed vesicular rash that is typical of varicella after an incubation period of 10–21 days. This pattern probably reflects viral spread to the tonsils and other local lymphoid tissues, from where infected T cells can transport the virus via the bloodstream to the skin[2] (FIG. 1a). During primary infection, virions presumably gain access to the sensory nerve cell bodies in ganglia by retrograde axonal transport from skin sites of replication or by T cell viraemia, and latent infection is established[3]. When viral replication is reactivated, VZV reaches the skin via anterograde axonal transport to cause the symptoms of zoster, which is characterized by a vesicular rash in the dermatome that is innervated by the affected ganglion. Both varicella and zoster skin lesions contain high concentrations of infectious virus and are thus responsible for transmission to susceptible individuals. Varicella epidemics occurred annually in the United States until a varicella vaccine (which is a live attenuated form of the VZV Oka strain) was introduced in 1995, but epidemics continue among children in countries that do not have immunization programs[4–6].

The VZV genome has at least 71 known or predicted ORFs[1,7] (BOX 1). Similar to all herpesviruses, VZV has a lipid-rich envelope, which is acquired from cellular membranes

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and into which viral glycoproteins are inserted. Within the envelope, a tegument layer that is predominantly composed of viral regulatory proteins surrounds an icosahedral nucleocapsid core that contains the linear double-stranded DNA genome[1]. The viral life cycle begins with VZV entry, which is a poorly understood process that is presumed to involve either direct fusion of viral particles with the plasma membrane or endocytosis (FIG. 1b). Viral envelope proteins are predicted to interact with cell surface molecules, such as mannose-6-phosphate receptor[8] or myelin-associated glycoprotein[9]. VZV glycoprotein B (gB), gH and gL function as the core fusion complex[9], but other envelope glycoproteins probably contribute as accessory proteins. After entry, the virions undergo uncoating, and tegument proteins, including the immediate-early protein 62 (IE62) — which is the major viral protein that functions as a transcription factor (that is, as a viral transactivator)[1,10] — are released and might be transported to the nucleus before *de novo* protein synthesis occurs. Nucleocapsids anchor at nuclear pores, where viral genomes are injected into the nucleus. VZV genome replication and viral gene expression depend on virus-encoded and host cell transcription factors and cellular translation systems[11]. Furthermore, the tegument proteins ORF47 and ORF66 are important serine/threonine kinases that autophosphorylate and phosphorylate viral transcription factors and other VZV proteins[12–18]. IE62 forms regulatory complexes with cellular factors, such as transcription factor specificity protein 1 (Sp1), which has binding sites in many viral promoters[11], to transactivate VZV genes. Similarly to other herpesviruses, nucleocapsids undergo primary envelopment, fusion with nuclear membranes and de-envelopment during transfer to the cytoplasm (FIG. 1b). Secondary envelopment occurs in the cisternae of the *trans*-Golgi network (TGN), where the capsids acquire tegument proteins and glycoprotein-containing membranes. Nascent virus particles then move to the cell surface in post-Golgi compartment vesicles; the first enveloped progeny virions are detected 9 hours after infection and many are present on cell surfaces within 12 hours of infection[19]. VZV differs from other herpesviruses in that assembled virions typically remain highly cell-associated. The same viral glycoproteins that are predicted to mediate entry are expressed on cell membranes and induce fusion of infected and uninfected cells, producing syncytia and multinucleated polykaryocytes, which further contributes to virus spread[20–25].

Box 1

VZV genome and virion structure

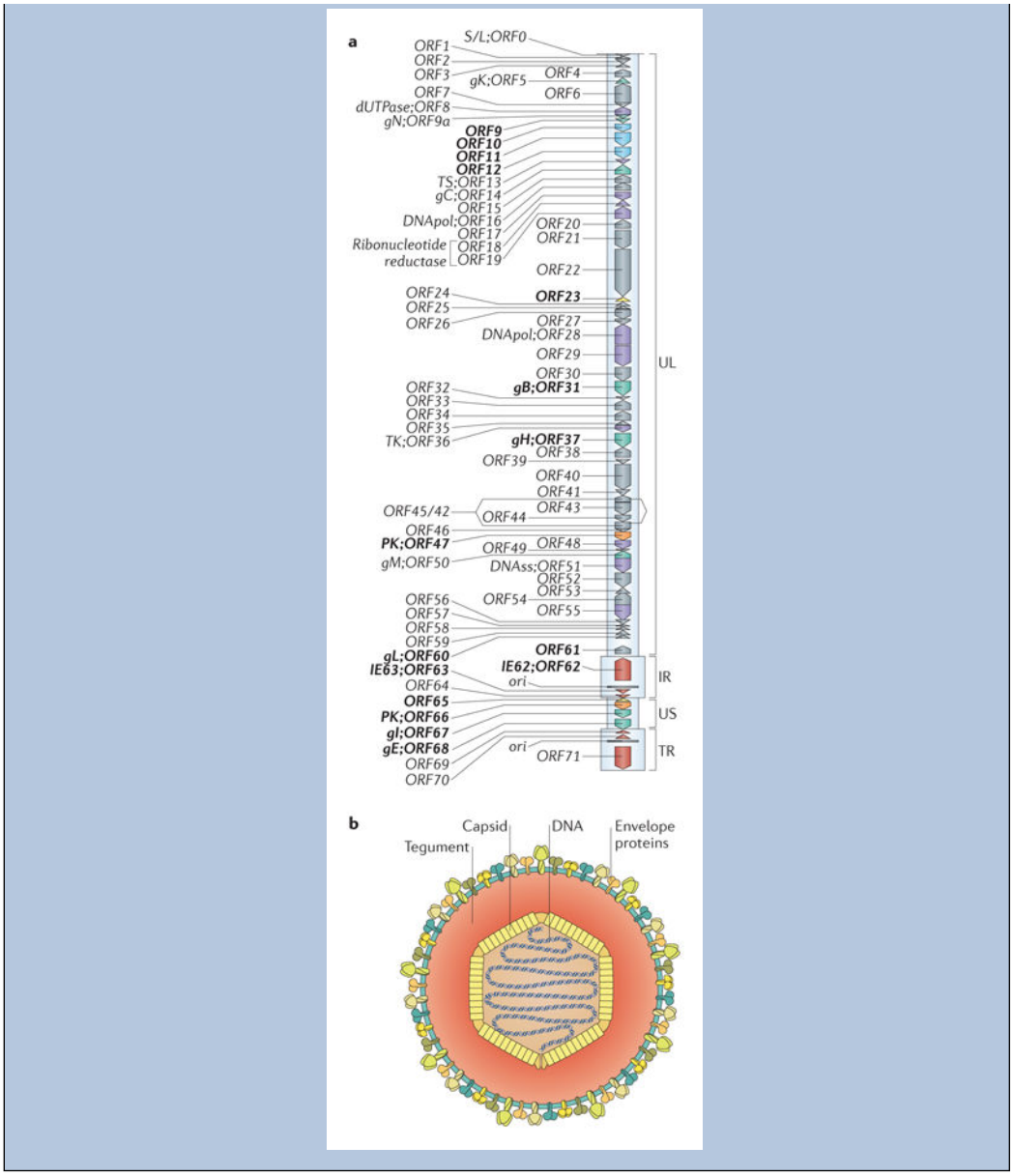
Genome structure

The VZV genome is a linear double-stranded DNA molecule of ~125,000 bp that encodes at least 71 unique ORFs and related promoter sequences[1,7]. Five phylogenetic VZV clades have been identified, but the most disparate still have 99.8% sequence conservation[69]. The genome consists of a unique long region (UL) of ~105,000 bp, a unique short region (US) of ~5,232 bp, and internal repeat (IR) and terminal repeat (TR) regions (see the figure, part **a**). The genes that encode ORF62 and ORF70, ORF63 and ORF71, and ORF64 and ORF69 are duplicated. The origin of replication (ori) is located in the repeat region. The VZV genome can circularize via unpaired bases at each end. About two-thirds of VZV ORFs are necessary for replication *in vitro*[77], most of which are among the ~40 genes that are conserved in all herpesviruses, including eight

glycoproteins (gB, gC, gE, gH, gI, gK, gL, gN; green), proteins that are involved in DNA replication (purple) and other functions, such as DNA cleavage and packaging, nucleic acid metabolism and capsid assembly. Replication proteins include the small and large subunits of the viral ribonucleotide reductase (known as ORF18 and ORF19), the two subunits of the viral DNA polymerase (known as ORF16 and ORF28), the single-stranded DNA-binding protein (known as ORF29), the origin of DNA replication binding protein (known as ORF51), two viral protein kinases (known as ORF47 and ORF66) and other enzymes that are involved in DNA replication, including dUTPase (known as ORF 8), thymidylate synthetase (known as ORF13), DNase (known as ORF48) and uracil DNA glycosylase (known as ORF59). Some VZV gene products have functional subdomains that are dispensable in cultured cells; others are dispensable for replication *in vitro* but are necessary for pathogenesis. The ORF9–ORF12 cluster of tegument proteins (blue) is conserved in the alphaherpesviruses. The products of the dispensable genes are of interest for their potential differential functions in tropism. Cloning the VZV genome into bacterial artificial chromosome vectors or as four or five overlapping fragments in cosmids enables the deletion of ORFs or targeted mutations of coding and non-coding sequences to define functions *in vitro* and *in vivo* (ORTs evaluated for pathogenesis indicated in bold, part a)[40,53,107,108].

Virion formation and structure

VZV particles are ~80–120 nm in diameter (see the figure, part **b**). Linear VZV genomes are packaged into an icosahedral nucleocapsid core that is formed from proteins encoded by *orf20*, *orf21*, *orf23*, *orf33*, *orf40* and *orf41* (REF. 1) Capsids are surrounded by a tegument layer, which is a less well-defined structure that is made up of proteins with known or predicted regulatory functions, including the immediate-early (IE) viral transactivating factors that are encoded by *orf4*, *orf62* and *orf63*, those that are encoded by the *orf9–orf12* gene cluster, the two viral kinases ORF47 and ORF66, and others. The outer virion component is a lipid membrane envelope that is derived from cellular membranes with incorporated viral glycoproteins, including gB/gH–gL, which form the minimal fusion complex.



Investigating VZV pathogenesis is challenging as VZV is a highly human-specific virus that has little or no capacity to infect other species. This obstacle can be overcome by using human tissue xenografts in mice with severe combined immunodeficiency (SCID) (BOX 2). Infecting foetal thymus-liver T cell, skin and dorsal root ganglia (DRG) xenografts enables studies of the three major tissue tropisms of VZV: T cell-, skin- and neuro-tropism[13,26,27]. In these models, innate responses that modulate infectious processes can be assessed independently of adaptive immunity, which is absent in SCID mice. VZV-specific T cells are necessary to clear primary infection and prevent symptomatic reactivation from latency, but the xenograft models show the importance of intrinsic responses of differentiated cells in the absence of an adaptive immune response. Such studies can be done in knockout mouse models that have defects in adaptive immunity, but VZV does not infect mice. Furthermore, the xenograft models have the advantage of investigating infection in the various human

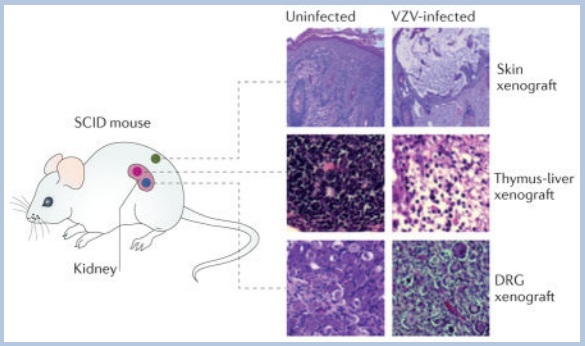
tissue microenvironments that are targeted by VZV. Inoculating human tissue xenografts with mutant VZV can show functions of viral genes that are dispensable in tissue culture but necessary under the more stringent conditions that are present in intact tissues and fully differentiated human cells *in vivo*. To investigate which host cell factors are required during infection, small-molecule inhibitors or antibodies that block cell functions can be administered. In this Review, we summarize the characteristics of the infectious process in T cells, skin and dorsal root ganglia, the contributions of VZV proteins and functional motifs within these proteins to the capacity of VZV to infect differentiated human cells (TABLE 1) and the modulation of VZV infection by host cell factors within the tissue microenvironment. Investigating pathogenesis using these tools offers insights into how VZV causes the clinical manifestations of varicella and zoster and how this ubiquitous virus has so successfully survived in the human population.

Box 2

Modelling the pathogenesis of varicella zoster virus infection

The pathogenesis of VZV infection can be modelled by infecting xenografts of human foetal tissues in mice with severe combined immunodeficiency (SCID)[26,27,33]. The xenografts are maintained for prolonged periods, as SCID mice lack T cell responses that mediate rejection of these foreign tissues. Skin xenografts are implanted beneath the mouse skin, and thymus-liver or dorsal root ganglia (DRG) xenografts are placed under the kidney capsule (see the figure). Then the human tissues become vascularized by anastomosis of capillaries in the xenografts with those in the surrounding murine tissues; endothelial cells of the microvasculature express human platelet endothelial cell adhesion molecule 1 (PECAM-1). Skin engraftment is established within 3–4 weeks; T cell and DRG xenografts require >8–12 weeks. Skin xenografts differentiate to have typical layers of dermal and epidermal cells, and epidermal keratinocytes differentiate into the anuclear corneocytes that comprise the stratum corneum, as in postnatal skin in humans. Thymus-liver xenografts contain immature dual-positive $CD4^+$ $CD8^+$ T cells but predominantly contain mature $CD4^+$ T cell and $CD8^+$ T cell populations. Neurons in DRG xenografts are surrounded by satellite cells, express neuronal subtype-specific proteins (such as TRKA and RT97), neural cell adhesion molecule (NCAM), synaptophysin and other markers such as herpes viral entry mediator (HVEM). After the period of engraftment, functional signalling pathways and effector proteins, including those that are involved in blocking apoptosis and innate antiviral responses (for example, signal transducer and activator of transcription (STAT) proteins, interferons and promyelocytic leukaemia protein (PML)) are present, as expected, in postnatal tissues. Effects of targeted mutations in the VZV genome are assessed by inoculating surgically exposed xenografts with fibroblasts that are infected with the parent wild-type or mutant virus, or with uninfected fibroblasts. The infectious process is similar when infected fibroblasts or when infected T cells — which are introduced into the mouse circulation — are used to deliver and release virions. To study the consequences of interfering with the functions of viral or cellular proteins, the mice are treated with antibodies or small-molecule inhibitors. Xenografts are recovered at different intervals after inoculation to assess viral titres, genome copies and viral transcripts; quantitative microscopy of tissue sections

shows viral and cellular protein expression, genome localization, virion assembly and ultrastructural changes in cells. Furthermore, progression of infection can be monitored *in vivo* using recombinant VZV that expresses firefly luciferase. The SCID mouse model also provides a system for translational research to assess live attenuated VZV vaccines and antiviral drugs[26,96,109].



T cell tropism

Discovering VZV tropism for T cells

VZV was initially classified as a neurotropic herpesvirus, but experiments using T cell xenografts in SCID mice *in vivo* and tonsil T cells *in vitro* have revealed that VZV also shows T cell tropism[13,26,28] (FIG. 2). $CD3^+$ T cells, including $CD4^+$, $CD8^+$ and dual $CD4^+CD8^+$ T cell subpopulations, are fully permissive for the replication and release of infectious virions. VZV infects tonsil T cells with high efficiency, which suggests that the virus is transferred from respiratory epithelial cells to T cells, presumably in the tonsils and other lymphoid tissues that comprise the Waldeyer's ring (FIG. 1), similarly to the transfer of Epstein–Barr virus to tonsil B cells[29]. VZV can also infect dendritic cells, which might facilitate spread to lymph nodes[30,31]. VZV-infected $CD4^+$ T cells predominantly show a memory T cell phenotype and express activation markers and skin-homing proteins, such as cutaneous leukocyte antigen (CLA) and CC-chemokine receptor 4 (CCR4), and are thus more likely to circulate through skin and other tissues[28]. In addition, VZV induces activation and skin homing proteins on naive T cells.

In SCID mice with skin xenografts, infected human tonsil T cells can transport VZV and initiate skin infection[2]. At 24 hours after injection of T cells into the circulation, infected T cells appear in the skin that surrounds hair follicles, where the capillary microvasculature is extensive, which indicates that these cells retain the ability to transit across capillary endothelial walls by diapedesis. Infected T cells also deliver VZV to DRG xenografts *in vivo*, which suggests that viraemia facilitates the establishment of latency[27]. VZV promotes survival of infected T cells by inducing signal transducer and activator of transcription 3 (STAT3), which gives these cells time to reach tissues[32]. Notably, VZV does not trigger fusion of infected T cells, which indicates that virions must enter each T cell separately[13,26,33]. This characteristic differs from the multinucleated syncytia that are formed in VZV-infected skin[34] and suggests that VZV has a cell type-specific capacity to suppress fusion, probably to retain the capacity of T cells to enter and exit tissues. The

ability of VZV to infect memory T cells without disrupting their trafficking explains the development of the scattered varicella lesions and is consistent with the cell-associated viraemia that is observed in clinical cases[35–37].

Viral determinants of T cell infection

The only glyco-proteins that are encoded by the short unique region of the VZV genome (BOX 1), gE and gI, have been investigated for their functions in T cells[33,38–40] (TABLE 1). In contrast to its homologues in other alphaherpesviruses, the VZV membrane glycoprotein gE has a large unique amino-terminal region (consisting of amino acids 1–188), which is essential for replication[39]. Within this region, gE amino acid residues 51–187 are important for secondary virion envelopment and T cell infection. Although gE typically forms a heterodimer with gI via a cysteine-rich ectodomain region at residues 208–236, this interaction is not needed to infect T cells[38,39]. In addition, amino acid residues 27–90 are required for binding to insulin-degrading enzyme (IDE), which is a cellular protein that contributes to VZV replication in melanoma cells *in vitro* and is a proposed entry receptor[41]. However, disrupting these interactions by mutating gE does not affect T cell-xenograft infection, which indicates that IDE is not needed for T cell entry. The gE cytoplasmic domain contains an endocytosis motif that is required for replication, whereas a TGN-targeting motif in this domain is dispensable for replication *in vitro* but is important in T cells *in vivo*[33]. VZV gI is required for T cell infection[42], and reducing gI synthesis by disrupting gI promoter sites for interactions with cellular transcription factors or ORF29 DNA-binding protein impairs replication in T cell xenografts[43].

Five of the tegument proteins that surround the viral capsid have been evaluated for their contributions to T cell infection, including ORF10 protein, IE63, ORF65 protein and the two viral kinases, ORF47 protein and ORF66 protein[13,16,17,44,45]. ORF10 protein increases IE62 expression but is not required for T cell infection[44]. IE63 also regulates IE62 expression and transactivates cellular elongation factor 1α (EF-1α)[49–52]: IE63 functions that are needed to infect T cell xenografts are preserved even when the extensive serine/threonine phosphorylation domain is disrupted[45]. The ORF65 protein is also dispensable[53]. In contrast, the VZV kinases have a major role in T cells: the kinase activity of ORF47 directs the intracellular localization of IE62, gE and ORF47 and is needed for virion assembly. Although these functions are dispensable *in vitro*[12,14,34], blocking ORF47 expression or disabling its kinase activity prevents VZV replication in T cell xenografts, which shows their importance *in vivo*[13]. Whereas ORF47 is conserved in all herpesviruses, only alphaherpesviruses have ORF66 kinase homologues[1,7]. ORF66 is important for virion assembly in differentiated T cells *in vivo*[17,18] and in human corneal fibroblasts but not in other cells cultured *in vitro*[15,18,46] (FIG. 1b). Furthermore, the ORF66 kinase inhibits apoptosis and counteracts the induction of interferon (IFN) signalling in infected T cells[17,18]. ORF66 also contributes to the downregulation of the major histocompatibility complex I and thereby interferes with $CD8^+$ T cell-mediated elimination of infected cells[47,48]. Thus, the ORF66 kinase, which is dispensable *in vitro*, has multiple functions in T cells *in vivo*.

Cellular transcription factors as determinants of T cell tropism

Investigating VZV mutants that have disrupted binding sites for cellular transcription factors shows the importance of the synergistic regulation of viral genes by IE62 and cellular cofactors. For example, the transcription factor specificity protein 1 (Sp1) increases the recruitment of IE62 to viral promoters[11,54], and mutation of the two Sp1-binding motifs in the gE promoter prevents VZV replication[55]. The gE promoter also has a site for upstream stimulatory factor (USF) binding, but it is not needed for the infection of tonsil T cells. Inhibiting the binding of Sp1 and USF to the gI promoter impairs VZV replication in T cell xenografts[43]. Thus, cellular cofactors that interact with IE62 have differential effects on particular viral gene promoters, which influence VZV replication in T cells *in vivo*.

Skin tropism

Innate cellular responses regulate skin pathogenesis. Lesion formation in infected skin xenografts is a highly regulated process that is determined by robust innate responses of epidermal and dermal cells (FIG. 3). Delivery of VZV to skin xenografts by infected T cells leads to the gradual formation of skin lesions over 10–21 days, which is consistent with the varicella incubation period[2]. Infected cells initially appear around hair follicles and viral proteins are then detected in clusters of adjacent cells, some of which fuse to create multinucleated polykaryocytes[37]. The uninfected cells that surround infectious foci show upregulation of interferon-α (IFNα) and IFNβ and the cellular transcription factors STAT1 and nuclear factor-κB (NF-κB), which orchestrate innate immune responses[2,56].

The importance of the IFNα and IFNβ response is evident from the enlarged lesions in skin xenografts that form if it is blocked with an antibody that targets the IFNα and IFNβ receptor[2]. During varicella in the human host, T cell-mediated immunity only occurs late in infection and is rarely detected until skin lesions have developed[1]. Adaptive responses are important to stop the infection[57], but the initial control of viral replication by innate responses probably contributes to the persistence of VZV in the population, as a severe infection that overwhelms the host would limit opportunities for VZV transmission to other susceptible individuals.

Notably, replication of the vaccine virus Oka is reduced in skin xenografts, which is consistent with the situation in humans, who rarely develop lesions after subcutaneous inoculation of the vaccine[4,5,26]. Thus, the mutations that have accumulated in this attenuated strain (via passage in fibroblasts) have reduced its capacity to overcome intrinsic cutaneous barriers, even though the mutations have no effect on T cell tropism[26] or neurotropism[5]. The Oka vaccine consists of a mixture of polymorphic viral clones, and some polymorphisms seem to be common, but none were identified as the molecular basis for attenuation. The evaluation of chimeric viruses constructed from segments of the pathogenic parental Oka strain and the attenuated vaccine in skin xenografts indicated that several genome regions contribute to viral attenuation in the skin[58], which is consistent with the presence of a mixture of VZV clones with varying sequence changes in Oka vaccine preparations[59].

VZV manipulation of cellular responses

VZV suppresses innate responses to produce a virus-filled lesion at the skin surface, where infected keratinocytes release newly-assembled virions[60] (FIG. 3). Several viral proteins interfere with IFN-mediated responses in infected cells: IE62 inhibits the phosphorylation of IFN regulatory factor 3 (IRF3) by TANK-binding kinase 1 (TBK-1), thus blocking IFNβ production[61], ORF47 kinase reduces IRF3 phosphorylation[62] and IE63 inhibits the phosphorylation of the eukaryotic initiation factor 2 and its downstream IFNα effects[63]. The ORF66 kinase inhibits the activation of STAT1, which is induced in response to IFNα and IFNβ signaling and upregulates IFN-stimulated genes (ISGs)[17]. VZV also sequesters the p50–p65 heterodimer, which is the most abundant form of NFκB, in the cytoplasm of epidermal cells, by preventing degradation of the NF-κB inhibitor-α (IκBα)[56]. The latter effect is cell type-specific, as VZV induces NF-κB in monocytes[48].

In addition to its inhibitory effects, VZV reprogrammes cell signalling to activate STAT3 in epidermal cells as well as T cells *in vivo* and fibroblasts *in vitro*[32]. Skin infection was severely impaired by treating SCID mice with a small-molecule inhibitor of STAT3, S31-201 (FIG. 3). STAT3-mediated upregulation of survivin, which is a cell protein that inhibits apoptosis, was necessary to support VZV infection. Although oncogenic herpesviruses manipulate this pathway to cause tumours, these experiments showed that VZV, which is a lytic herpesvirus, must also induce survivin. Interestingly, survivin expression is constitutive in the epithelial cells that line the hair follicles, which is the initial site of VZV replication in skin. VZV triggers autophagosome formation in skin cells, which might also reinforce the delay of apoptosis, thereby favouring infection[64].

Promyelocytic leukemia protein (PML) in the regulation of skin infection

PML is a multifunctional protein that has antiviral effects against many viruses and is upregulated by IFNs. In VZV-infected cells, PML can form intranuclear cages that trap nascent virions and restrict their egress from the nucleus to the cytoplasm[65,66]. This antiviral effect is mediated by PML isoform IV, which binds to the ORF23 outer capsid protein via a unique carboxy-terminal domain and sequesters virions.

PML is a predominant component of nuclear structures known as PML nuclear bodies (PML-NBs; also known as nuclear domain 10). PML-NBs are more abundant in skin than in cultured cells, and VZV infection and the concomitant IFN secretion cause further accumulation in uninfected, adjacent epidermal and dermal cells, thus potentially limiting viral replication *in vivo*[67] (FIG. 3). The ORF61 protein, which is expressed very early after infection[19], functions to counteract this antiviral effect by binding to PML via SUMO-interacting motifs (SIMs) and disrupting the architecture of PML-NBs (FIG. 3). Mutation of the ORF61 SIMs has no consequences *in vitro*, but, in skin, it enables PML-NBs to remain abundant, severely impairs VZV replication and prevents viral penetration of the cutaneous basement membrane[67]. Again, IE62 synergism with Sp1 is necessary, as interfering with the binding of Sp1 to the ORF61 promoter limits ORF61 expression and reduces the extent of skin lesions[68].

Cell–cell fusion and skin tropism

Cell–cell fusion is not strictly required for VZV spread, as virions that are released from infected cells can enter adjacent cells[19]. However, polykaryocyte formation is the classic pathological change that is induced by VZV in the skin. This pattern suggests that VZV reprogrammes infected cells to overcome the normal preservation of plasma membrane boundaries between differentiated cells and to mediate fusion of human skin cells *in vivo*, leading to facilitated virus spread, which overcomes innate barriers.

Glycoprotein gB and the heterodimer that is formed by gH and gL constitute the minimal VZV fusion complex and are candidates for mediating virus-induced cell fusion as well as virion entry[9,20,23,24] (FIG. 1b; TABLE 1). The gB ectodomain has a highly conserved primary fusion loop that is essential for replication, and a furin protease recognition motif, which is not found in most alphaherpesvirus homologues[24]. Disrupting furin cleavage attenuates viral infection of skin xenografts, which indicates that this post-translational modification of gB is important for pathogenesis. According to the current model, herpesvirus gH (together with gL) activates gB to trigger its fusogenic function. For VZV, this function seems to depend, in part, on gH ectodomain residues and is required for skin pathogenesis, as administering a gH-specific monoclonal antibody blocks replication in skin xenografts[22]. Residues in the extreme amino-terminus of gH (which are dispensable *in vitro*) also contribute to skin infection *in vivo*[23].

Although cell–cell fusion is a prominent characteristic of VZV pathogenesis, it must be tightly controlled during skin infection. Appropriately regulated cell–cell fusion depends on the cytoplasmic domain of gB and is mediated by a previously unrecognized immunoreceptor tyrosine-based inhibition motif (ITIM) in the gB cytoplasmic domain[25] (FIG. 3). Phosphorylation of tyrosine 881 (Y881) in the ITIM modulates VZV-induced fusion, and blocking ITIM phosphorylation leads to a hyperfusogenic phenoptye *in vitro* and reduces the production of infectious virions. Inhibition of ITIM phosphorylation *in vivo* causes the aberrant fusion of skin cells in the outermost layer and markedly impairs lesion formation and infectious virus yields, which suggests that the gB cytoplasmic domain is essential for the control of polykaryocyte formation and optimal skin infection.

Glycoproteins gE and gI as determinants of skin infection

Although VZV isolates can be classified into several distinct clades that reflect their geographical origin, VZV is genetically stable, and unrelated isolates exhibit little variability in virulence[69]. However, VZV-MSP, which is a naturally occurring variant with a single amino acid change (D150N) in the gE ectodomain, has increased cell–cell spread *in vitro* and accelerated growth in skin xenografts compared with other isolates[70]. Highlighting the potential effect of changing a single gE residue, a serine to alanine substitution at gE position 31 markedly impairs skin replication[39].

Residues 51–187 in the non-conserved ectodomain of gE are essential for both skin tropism and T cell tropism[39], which reinforces the importance of this unique gE region for the VZV life cycle (TABLE 1). Blocking gE–gI heterodimerization interferes with gE maturation and surface expression and inhibits the incorporation of gI into virions. These functions that

involve gE–gI-binding are important for skin tropism but are dispensable in T cells[38]. Binding of gE to IDE also contributes to skin tropism, but blocking the interaction of gE with this cellular protein has much less effect than eliminating gE–gI binding. Overall, gE domains, including the TGN-targeting motif in the C-terminus of gE[33], are more important for the infection of skin than of T cells, which suggests that gE functions determine cell–cell spread rather than initial virion entry. Binding sites for cellular factors in the gE promoter, other than Sp1, are not necessary for replication or skin tropism[55].

Deleting gI interferes with gE trafficking and secondary envelopment of virions *in vitro* and completely blocks infection of skin and T cells *in vivo*, which can be attributed to defective virion assembly[41,71]. Ensuring that gI expression levels are sufficient for normal skin pathogenesis depends on the enhancing effect of the ORF29 DNA-binding protein for IE62 transactivation[43]. Co-regulation of the gI promoter by Sp1 and USF with IE62 is essential in skin, whereas it enables a low level of replication in T cell xenografts[43], which indicates tissue-specific differences in the requirement for cell transactivators that influence levels of gI expression.

Tegument and capsid proteins in skin infection

Like other herpesviruses, the VZV genome has duplicate copies of genes in the repeat regions, including those that encode the tegument proteins, IE62 (encoded by *orf62* and *orf71*), IE63 (encoded by *orf63* and *orf70*) and ORF64 protein (encoded by *orf64* and *orf69*)[1,7] (BOX 1). The importance of this genomic organization for pathogenesis is not known. Deleting the genes for IE62 or IE63 is lethal for VZV replication but the *orf64–orf69* gene pair is dispensable, which indicates that gene duplication is not conserved to protect against the loss of an essential gene[50]. However, when skin xenografts are infected with VZV mutants that have single copies of the genes for IE62 and IE63 genes, the second copy is restored by recombination during replication, which suggests that gene duplication is important *in vivo*[45,72]. IE62 production in skin is necessary, as is evident from impaired infection when the binding site for the ORF29 viral cofactor is mutated in the *orf*62–*orf70* promoter. A single copy of *orf63* at a non-native site in the genome provides sufficient IE63 for replication in skin; however, lesion formation is substantially impaired if IE63 phosphorylation is disrupted, which indicates that there are differential requirements for the IE63 phosphoprotein in skin and in T cells[45].

The VZV tegument contains proteins that are encoded by a cluster of genes (known as *orf9–orf12*) that is conserved in alphaherpesvirus genomes[7]. Of these genes, only *orf9* is essential *in vitro*. The ORF10 protein increases expression of IE62 and is important for skin infection, which shows that interactions of IE62 with viral cofactors — which are dispensable *in vitro* — are necessary for pathogenesis *in vivo*[44,73]. The ORF11 protein contributes essential functions in skin xenografts via its binding to the ORF9 protein during virion tegument assembly; however, the capacity of the ORF11 protein to bind to mRNA is not necessary, which shows that distinct functions of the same VZV protein differ in their effects on skin tropism[74]. The ORF12 protein — which activates ERK1/2, inhibits apoptosis[75] and manipulates cell cycle progression by activating the PI3K/AKT pathway[76] — is dispensable in skin[73]. Thus, although functions of some gene products are essential, conservation of the

orf9–12 cluster does not signify a uniform requirement of these genes for pathogenesis in differentiated human tissues.

Disrupting the ORF47 protein kinase function, which causes the nuclear retention of ORF47 and IE62 proteins *in vitro*, severely impairs skin pathogenesis[14,34]. Deleting *orf47* also reduces replication in skin organ cultures[77]. However, despite reduced virion formation, the ORF47 kinase mutant elicits some polykaryocyte formation in skin xenografts as long as binding of the ORF47 protein to IE62 is preserved[34]. By contrast, replication of the ORF47 kinase mutant in T cell xenografts is completely blocked, which suggests that even minimal cell fusion can support VZV spread in the skin, whereas T cell infection depends on the complete assembly and release of infectious virions[13,14]. Notably, disabling ORF66 kinase activity has very little effect on virulence in the skin, despite its importance for T cell tropism[13]. Thus, the ORF47 and ORF66 viral kinases make different and tissue-specific contributions to VZV pathogenesis.

The small capsid protein that is encoded by *orf23* is dispensable for replication *in vitro*, owing to redundant ORF33.5-mediated transport of the major capsid protein into the nucleus[78]. However, this mechanism is not sufficient in skin, which indicates that there are strict requirements for VZV capsid assembly *in vivo*.

Neurotropism

Before the DRG-xenograft model was developed, VZV neurotropism in human tissues could only be examined in sensory ganglia that were obtained at autopsy. These studies showed that VZV genomes (~2–9 copies per cell) are present in about 4% of neurons during latency[79,80]. Transcripts of ten VZV genes have been reported in autopsy ganglia, and *orf63* transcripts are the most abundant[81,82], but the extent to which detection represents post-mortem release of gene silencing is not known. In some studies, VZV proteins were rare or absent, whereas others reported frequent expression in the neuronal cytoplasm[83,84]; however, eliminating an artefact, which results from antibody reactivity against blood group A determinants, confirms that VZV protein expression is rare in latency[85]. By contrast, ganglia from individuals who had zoster a few months before death harbour VZV proteins and inflammatory proteins in up to 25% of neurons[86,87]. Prolonged replication in ganglia might be a factor in some cases of postherpetic neuralgia.

VZV infection and latency in DRG xenografts

In DRG xenografts, viral proteins, genomes and infectious virus are detectable for 3–4 weeks after VZV inoculation[27] (FIG. 4). Productive infection is followed by a transition to VZV latency after 4–8 weeks. During this phase, infectious virus is no longer detectable, viral genome copies decline, levels of *orf62* and *orf63* transcripts are reduced and gB transcription ceases. This pattern of DRG infection is identical after direct inoculation or VZV transfer from infected T cells. The transition to persistence in neurons occurs despite the absence of adaptive immunity and markedly contrasts with the progressive lytic infection that is observed in skin and T cell xenografts. Thus, VZV gene silencing in neurons is a cell type-specific characteristic of the VZV–host interaction. Infection of

explanted foetal DRG neurons *in vitro* showed that IE63 has anti-apoptotic functions that might facilitate this transition[88,89].

As observed in skin xenografts, VZV infection of DRG is regulated by the capacity of PML-NBs to sequester nucleocapsids in neurons and satellite cells[65]. Cages that consist of PML fibres surround both nascent and fully formed virions in infected cell nuclei. Notably, neurodegenerative disorders, such as Huntington's disease, are also associated with large PML-NBs that retain aberrant polyglutamine proteins. PML isoform IV cages have the capacity to sequester both viral capsids and the aberrant Huntington's disease protein, HttQ72 (REF. 90), which suggests that neurons have a conserved mechanism to sense and entrap protein aggregates.

How VZV reactivation is triggered is not known, but intrinsic cellular mechanisms might also restore gene silencing, resulting in abortive replication. If replication continues, VZV is transported to skin, causing zoster (FIG. 1). VZV-specific T cells then function together with innate cutaneous responses to control the infection. VZV cell-mediated immunity is deficient in elderly and immunocompromised patients, which accounts for more frequent and severe zoster in these populations[92]. Whether VZV-specific T cells also limit reactivation in ganglia is not known.

Neuron–satellite-cell fusion

When VZV replicates in DRG xenografts, VZV genomic DNA, viral proteins and virion production are detectable in both neurons and satellite cells[27,92]. Importantly, VZV can induce fusion and polykaryocyte formation between differentiated neurons and surrounding satellite cells (FIG. 4), whereas herpes simplex virus 1 (HSV-1) does not induce cell fusion in DRG[92,93]. Neuron–satellite cell fusion is reported in ganglia from patients with zoster at the time of death[94], which indicates that DRG xenograft infections are a model of this consequence of VZV reactivation. This formation of neuron–satellite-cell polykaryons amplifies the spread of VZV to neuronal cell bodies in the ganglion. As VZV is transported to the skin by neuronal axons that extend from neuronal cell bodies, spread within ganglia would increase the extent of infection of the dermatome during zoster. These pathological changes are not readily reversible and help to explain why zoster can be associated with prolonged neuropathic pain[1].

Glycoproteins gE and gI as determinants of neurotropism

Both gE and gI have functions that influence VZV pathogenesis in DRG xenografts (TABLE 1). Infection is not altered when binding of gE to IDE is disrupted, although neural cells express IDE, and interference with the TGN-trafficking motif of gE also has no effect on neurotropism[95]. By contrast, blocking gE–gI heterodimer formation impairs cell–cell spread in DRG and reduces replication during acute infection, with no infectious virus detected until four weeks after inoculation. However, instead of transitioning to persistence, the gE mutant causes widespread cytopathic changes in neurons, satellite cells and the surrounding tissue, for at least two months after inoculation[96,97]. Thus, the gE–gI interaction is crucial for preventing a chronic, highly destructive process in sensory ganglia.

Deleting gI, similarly to blocking heterodimer formation, results in the prolonged production of infectious virus particles despite reduced virion assembly and gE mislocalization[97]. Impaired virion assembly is associated with the accumulation of aberrant membrane stacks in the TGN region and altered Golgi structures both in DRG neurons and *in vitro*[98]. These effects, like those that are observed when gE cannot bind to gI, might be due to the absence of cell fusion, such that the virus can only spread slowly from cell to cell and host innate responses that faciliate the transition to persistence are less effectively triggered. Of interest, gI promoter regulation by Sp1 and USF is not required for neuropathogenesis[97], in contrast to T cell and skin infection, which, again, indicates that cellular factors contribute in a cell type-specific manner to viral gene expression and therefore to tissue tropism. Surprisingly, the ability of VZV to replicate in DRG in the absence of gI suggests that the requirements for VZV infection of neural cells are less stringent than those that are required for infection of T cells or the skin. Finally, the unexpected finding that disrupting the formation of gE–gI heterodimers, or deleting gI, causes chronic infection highlights the need to evaluate effects on neurovirulence *in vivo* and shows that the consequences of VZV mutations cannot be predicted from their effects in other tissue microenvironments.

Perspective

These studies in the SCID mouse model show that virus–host cell interactions result in a well-regulated infectious process in each of the tissue microenvironments that is important for VZV pathogenesis. In infected cells, the virus reprogrammes cell signalling pathways by inducing or downregulating cellular factors, such as the STATs, to support replication. VZV also has tissue-specific effects that are important for pathogenesis, such as suppressing the fusion of infected T cells. Although many viral proteins are incidental for VZV replication *in vitro*, specialized functions, not only of complete VZV proteins but also small motifs, as well as single amino acids, are often crucial for pathogenesis *in vivo*. To protect these crucial functions, VZV has a high degree of genome stability[69]. At the same time, innate responses of uninfected cells modulate infection so that the host is not overwhelmed, which benefits both the host and the virus by ensuring that there are opportunities for transmission and persistence in the host population.

Despite these advances in understanding VZV–host interactions, many questions about VZV pathogenesis remain unresolved; for example, as is the case for other herpesviruses, the triggers of VZV reactivation from latency are unknown. DRG xenografts can be used to investigate VZV–neuron interactions, but axonal transport to skin cannot be examined in this model, and stimuli of viral reactivation from latency have not been explored. By contrast, simian varicella virus (SVV) can be studied in the natural non-human primate host[99,100] and has been used to investigate reactivation that is associated with immunosuppression[84]. The SVV model avoids the need to acquire human tissue to make xenografts, which is a limitation of the SCID model, but genetic dissimilarities between SVV and VZV are likely to lead to differences in pathogenesis in their native hosts. Mechanisms of neuropathic pain cannot be assessed in DRG xenografts, but they can be investigated in the rat footpad model[101], and infection of enteric neurons has recently been reported in guinea pigs[102]. Although cultured neurons lack the surrounding satellite cells that mediate neuronal homeostasis *in vivo*, viral protein functions of interest for further

study *in vivo* can be identified in neuron cultures, as was shown for the ORF7 protein[103]. Although the xenograft models are valuable for probing intrinsic antiviral defences, advances in establishing a human immune system in SCID mice might make it possible to assess adaptive immune clearance of VZV in conjunction with innate control.

From a clinical perspective, VZV remains a medically important human herpesvirus despite major advances in vaccines and antiviral drugs to prevent or mitigate VZV infection. Live attenuated VZV vaccines are effective in healthy individuals but are not safe for immunocompromised patients, in whom they cause viraemia, and they can establish latency and reactivate in healthy and in immunodeficient individuals[4–6,104,105], which is consistent with the lack of attenuation that is observed in T cell and DRG xenografts. The genetic basis of their attenuation is not defined[58,59]. The SCID mouse model can be exploited for rational VZV vaccine design by incorporating mutations that dampen replication in skin into the viral genome, similarly to the current vaccine, and, in contrast to the current vaccine, that also interfere with the capacity to infect T cells or to persist in neurons. Antiviral drugs, such as acyclovir and related agents, substantially reduce the risk of severe or fatal VZV infection in immunocompromised patients but have little or no effect on postherpetic neuralgia following zoster in the elderly[106]. Knowledge about functional motifs of VZV proteins and how the virus reprogrammes differentiated human cells *in vivo* might help in designing small-molecule inhibitors with antiviral activity that would also decrease postherpetic neuralgia. Understanding the principles of VZV pathogenesis at the molecular level has the potential to yield new approaches to prevent and treat VZV infections.

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Glossary

Dermatome	An area of skin that is primarily innervated by a single sensory ganglion of the spinal cord
***Trans*-Golgi network (TGN)**	An intracellular collection of tubules and vesicles that are located at the *trans* face of the Golgi stack; it is involved in processing glycoproteins
Syncytia	Multinucleated cells that are created by fusion of membranes between cells with single nuclei; multinucleated cells in tissue are referred to as polykaryocytes
Polykaryocytes	Cells that contain many nuclei
Xenografts	Tissues from one species that have been transplanted into a different species

Severe combined immunodeficiency (SCID)	A genetic defect that blocks the function of T cells and B cells, interfering with the capacity of the host to mount an effective immune response to foreign proteins
Dorsal root ganglia (DRG)	Nodules on the dorsal root of the spinal cord that contain sensory nerve cell bodies
Anastomosis	The end-to-end connection of tubular structures, such as capillaries
Waldeyer's ring	An annular arrangement of lymphoid tissue in the oropharynx; it consists of the pharyngeal, tubal, palatine and lingual tonsils
Diapedesis	The passage of lymphocytes and cells of the immune system through intact vessel walls
Major histocompatibility complex I	A protein that is present on the surfaces of most mammalian cells that functions to present peptide epitopes to T cells
Satellite cells	Glial cells that surround the surfaces of neurons in the peripheral nervous system
Neuropathic pain	Pain that is caused by damage to the somatosensory system; it is associated with increased sensitivity to touch, temperature and other stimuli

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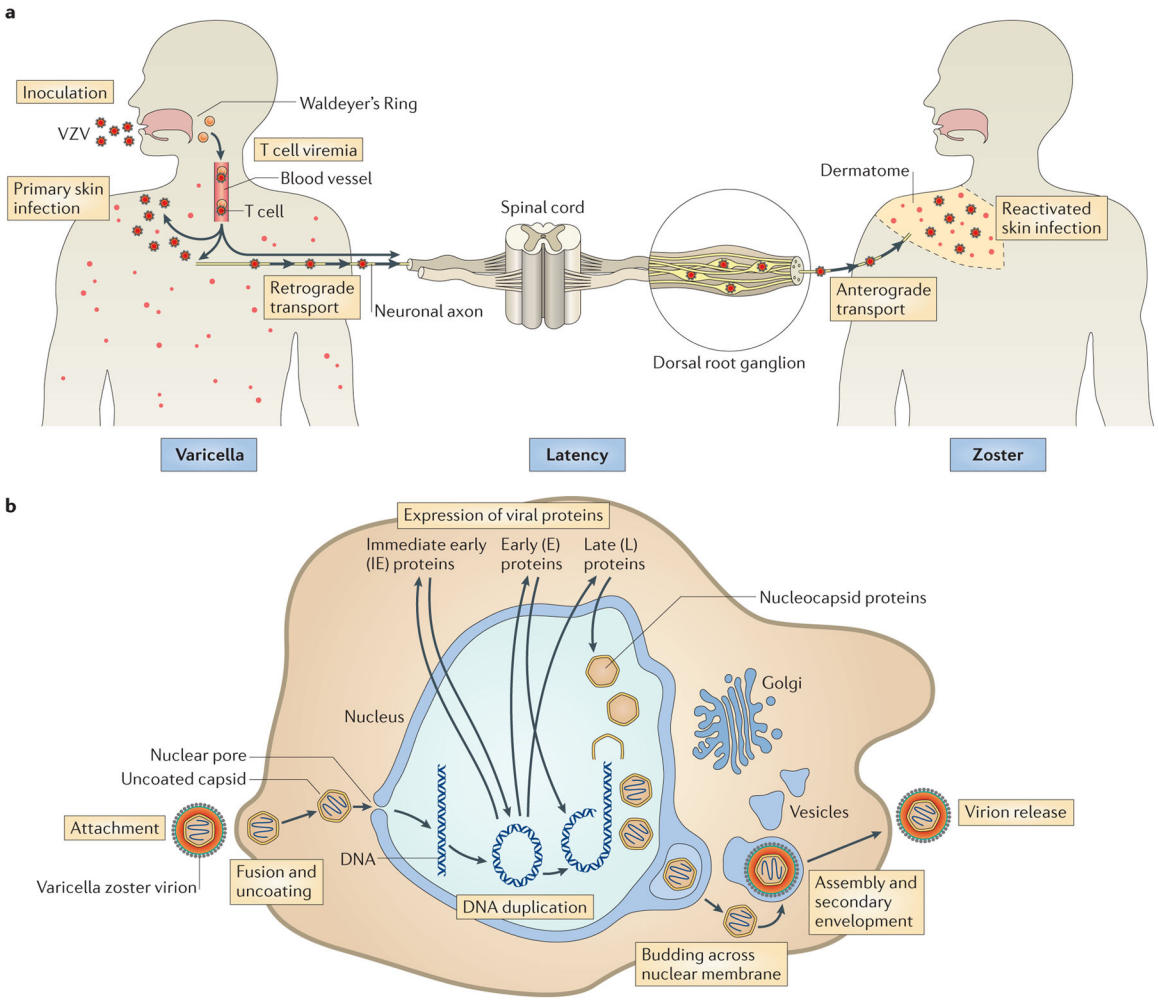

Figure 1. VZV life cycle and replication

a | Model of the varicella zoster virus (VZV) life cycle. VZV infects the human host when virus particles reach mucosal epithelial sites of entry. Local replication is followed by spread to tonsils and other regional lymphoid tissues, where VZV gains access to T cells. Infected T cells then deliver the virus to cutaneous sites of replication. VZV establishes latency in sensory ganglia after transport to neuronal nuclei along neuronal axons or by viraemia. Reactivation from latency enables a second phase of replication to occur in skin, which typically causes lesions in the dermatome that is innervated by the affected sensory ganglion. **b** | Model of VZV replication. Enveloped VZV particles attach to cell membranes, fuse and release tegument proteins. Uncoated capsids dock at nuclear pores, where genomic DNA is injected into the nucleus and circularizes. On the basis of events that have been documented in herpes simplex virus 1 (HSV-1) replication, immediate-early genes are expressed, followed by early and late genes. Nucleocapsids are assembled and package newly synthesized genomic DNA, move to the inner nuclear membrane and bud across the nuclear membrane. Capsids enter the cytoplasm, and virion glycoproteins mature in the *trans*-Golgi region and tegument proteins assemble in vesicles; capsids undergo secondary

envelopment and are transported to cell surfaces, where newly assembled virus particles are released.

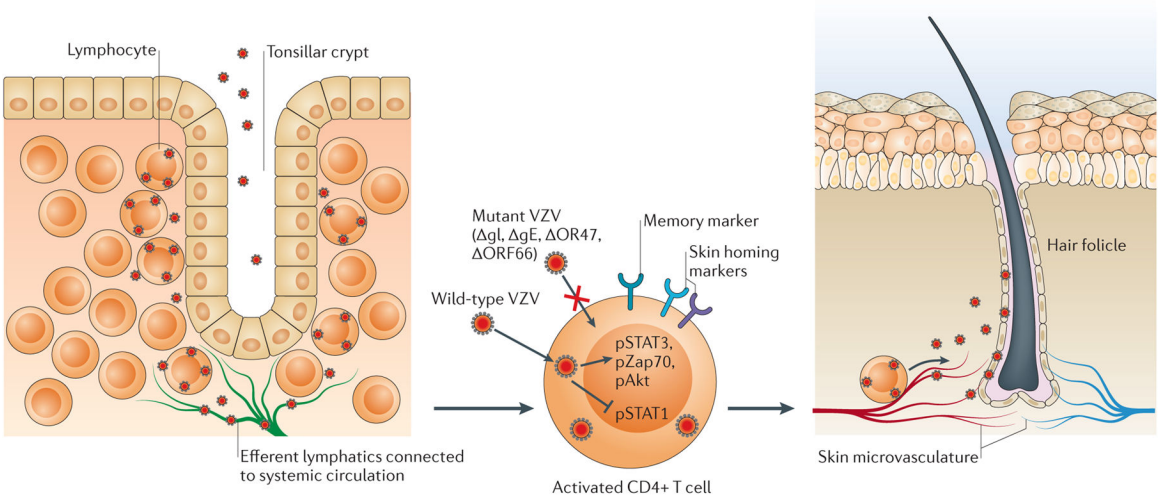

Figure 2. VZV T cell tropism

According to the model of varicella zoster virus (VZV) cell-associated viraemia, tonsil T cells are infected following VZV inoculation and replication in respiratory mucosal epithelial cells. T cells traffic into and out of tonsils across the squamous epithelial cells that line the tonsilar crypts (left panel). VZV has increased tropism for activated memory T cells that have skin-homing markers, which are common in tonsils (centre panel). These T cells are programmed for immune surveillance and can transport the virus across capillary endothelial cells into skin. VZV glycoprotein E (gE) (through its unique amino terminus), gI and the viral kinases ORF47 and ORF66 are important for T cell infection. Proteins that regulate cellular gene expression are activated (in the case of signal transducer and activator of transcription 3 (STAT3)) or inhibited (in the case of STAT1) in infected T cells. The microvasculature is extensive at the base of hair follicles, where T cells transit into the surrounding skin and initial VZV replication is observed (right panel).

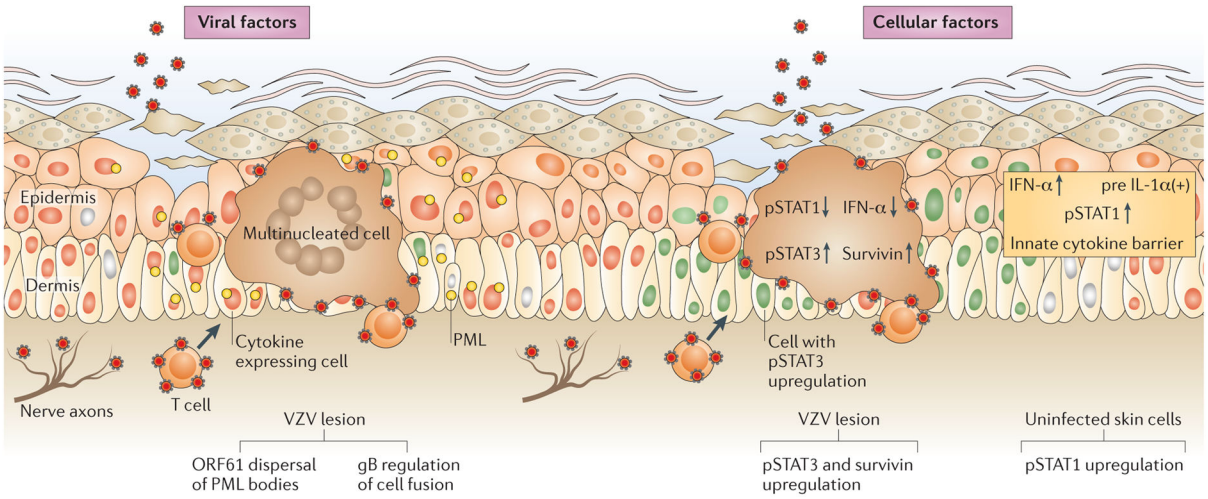

Figure 3. VZV skin tropism

The schematic illustrates viral factors that ensure spread to the skin surface after varicella zoster virus (VZV) is delivered to cutaneous sites of replication by infected T cells or by retrograde axonal transport from neurons (left-hand side). Two examples of VZV proteins that are important for pathogenesis are shown: ORF61 protein has SUMO-interacting motifs that are important for dispersal of promyelocytic leukaemia nuclear bodies (PML-NBs)[67] and the cytoplasmic domain of glycoprotein B (gB) has an immunoreceptor tyrosine-based inhibition motif that regulates cell–cell fusion and polykaryocyte formation[25]. VZV replication in skin triggers cellular responses, including changes that are induced in infected cells and changes in the uninfected cells adjacent to infected cells. Examples of VZV effects within infected cells are illustrated (right-hand side). VZV induces signal transducer and activator of transcription 3 (STAT3) activation, which triggers the expression of the anti-apoptotic protein survivin and inhibits the expression of interferon-α (IFNα) and STAT1 (REF. 32). In contrast to infected cells, surrounding uninfected cells exhibit upregulation of IFNs, STAT1, which activates IFN-stimulated factors such as PML, and other cell transacivators and innate cytokines[2].

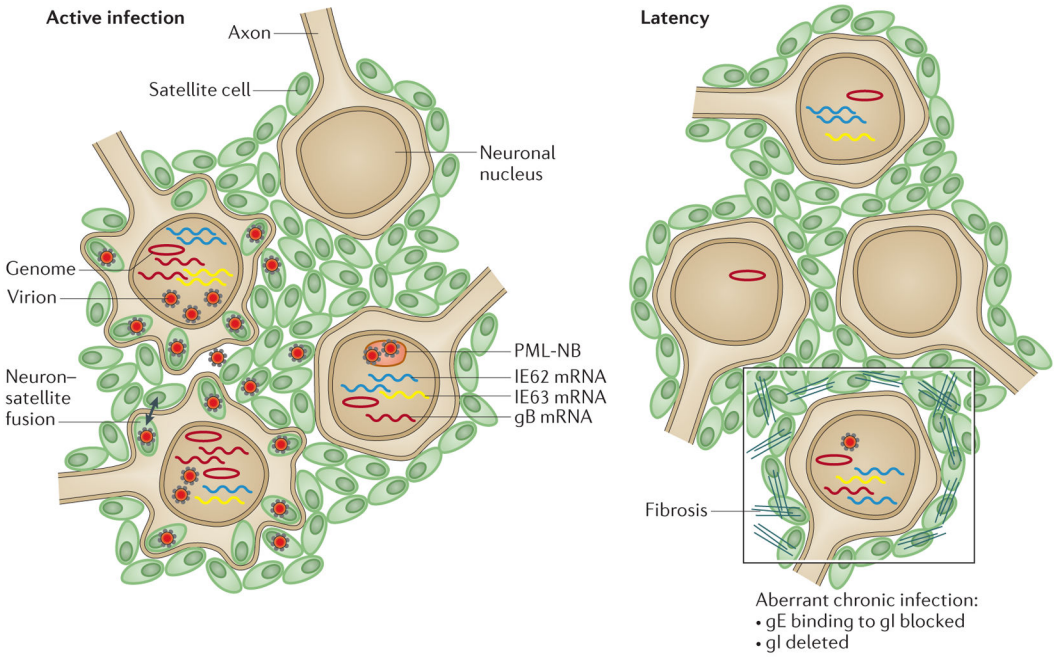

Figure 4. VZV neurotropism in DRG xenografts

This schematic illustrates active infection of dorsal root ganglia (DRG) which is characterized by the transcription of genes (for example, genes encoding glycoprotein B (gB), immediate early protein 62 (IE62) and IE63) that produce proteins that are required for lytic infection, varicella zoster virus (VZV) genome synthesis, virus assembly in neurons and satellite cells, release of VZV into intracellular spaces and fusion of some neurons and satellite cells[27] (left panel). Virions are captured in cages that are formed by promyelocytic leukaemia nuclear bodies (PML-NBs) in some neurons and satellite cells[66]. By contrast, latency (right panel) is associated with the persistence of VZV genomes and immediate-early (IE) transcripts, whereas late gene transcription, such as transcription of gB, ceases and virion formation ceases. When DRG are infected with VZV mutants in which binding of gE to gI is blocked or in which gI is deleted, the transition to latency is disrupted (right panel; outlined box), infectious virions continue to be produced at low levels and in the case of disrupted binding of gE to gI, tissue destruction is extensive, which is associated with disruption of the cell matrix, elimination of many neurons and the proliferation of satellite cells.

Table 1

VZV protein functions in the pathogenesis of T cell, skin and DRG infection

Protein	Characteristics	Cell culture*	Xenografts*
Glycoproteins			
gB (ORF31)	Mature form generated by furin cleavage; ITIM motif in cytoplasmic domain; required for cell fusion together with gH–gL	Essential	Mutation of furin-cleavage site: impaired replication in skin
			ITIM mutation; severely impaired replication and enhanced fusion in skin
gH (ORF37)	Ectodomain comprising domains I–III; forms heterodimer with gL; required for cell fusion together with gB and gL	Essential	Mutation of domain I amino terminus: impaired replication in skin
			Mutation of domain III fusion loop; impaired replication in skin
gL (ORF60)	Forms heterodimer with gH	Presumed essential	Not tested
gE (ORF68)	Large unique N terminus (amino acids 1–187); forms heterodimer with gI (amino acids 208–236); binds to IDE; TGN-targeting and endocytosis motifs in cytoplasmic domain; MSP−gE is a natural variant	Essential; deletion of amino acids 1–187 blocks endocytosis	Deletion of amino acid residues 51–187: no replication in T cells and skin
			Mutation of gI-binding domain: no effect in T cells, severely impaired replication in skin, prolonged replication and severe tissue damage in DRG
			Mutation of IDE-binding domain: no effect in T cells, impaired replication in skin, no effect in DRG
			Mutation of TGN-targeting motif: impaired replication in T cells and skin, no effect in DRG
			MSP-gE: enhanced replication in skin
gI (ORF67)	Forms heterodimer with gE (via amino acids 105–125 and conserved cysteine residues); required for incorporation of gE into virions	Dispensable, decreased titres and plaque size	Deletion of gI: no replication in T cells and skin, prolonged replication in DRG
			Mutation of gE-binding domain: prolonged replication in DRG
			Deletion of amino acids 105–125: no replication in skin
			Mutation of Sp1–USF-binding motif: no replication in T cells, impaired replication in skin, no effect in DRG
			Mutation of ORF29-binding motif: impaired replication in T cells
Regulatory proteins			
ORF9	Tegument protein; binds to IE62 and ORF11; functions unknown	Essential	Not tested
ORF10	Tegument protein; ORF62 and ORF71 transactivator; required for efficent virion assembly	Dispensable	Deletion of ORF10: no effect in T cells, impaired replication in skin
ORF11	Tegument protein; required for normal levels of IE4, IE62, IE63 and gE; RNA-binding domain (amino acids 1–22); binding to ORF9 required for efficient virion assembly	Dispensable	Deletion of ORF11: impaired replication in skin
			Mutation of RNA-binding motif: no effect in skin

Protein	Characteristics	Cell culture*	Xenografts*
			Mutation of ORF9-binding motif: no replication in skin
ORF12	Tegument protein; activates cell signalling pathways (such as ERK, p38, JNK and PI3K–AKT) that inhibit apoptosis and support viral replication	Dispensable	Deletion of ORF12: no effect in skin
ORF47	Serine/threonine kinase (conserved); phosphorylates regulatory proteins and glycoproteins; binds to IE62; required for virion assembly	Dispensable	Deletion of ORF47: no replication in T cells and skin
			Mutation of kinase motif: no replication in T cells, impaired replication in skin
ORF61	Transactivator or repressor; dimerization required for regulatory functions; E3 ligase; PML dispersal by SIMs	Essential‡	Deletion of SIMs: impaired replication in skin
			Deletion of dimerization domain (amino acids 250–320): impaired replication in skin
ORF62 and ORF71	IE62 is major viral transactivator; IFN inhibition	Essential	Deletion of ORF62 and ORF71 with ectopic ORF62: no replication in skin
ORF63 and RF70	IE63 phosphoprotein; represses IE62; transactivates EF−1α	Essential‡	Deletion of ORF63 and ORF70 with ectopic ORF63: no effect in T cells and skin
			Mutation of phosphorylation sites: no effect in T cells, impaired replication in skin
ORF66	Serine/threonine kinase (alphaherpesviruses); phosphorylates IE62; inhibits apoptosis	Dispensable	Deletion of ORF66: impaired replication in T cells, slightly impaired replication in skin
			Mutation of kinase motif: impaired replication in T cells, slightly impaired replication in skin
Other VZV proteins			
ORF23	Small capsid surface protein; required for nuclear transport of other capsid proteins and capsid assembly	Dispensable	Deletion of ORF23: no replication in skin
ORF35	Cell fusion	Dispensable, decreased cell fusion	Deletion of ORF35: slightly impaired replication in T cells, impaired replication in skin
ORF64 and ORF69	Cell fusion	Dispensable, increased cell fusion	Deletion of ORF64 and ORF69: no effect in T cells and skin
ORF65	Virion protein	Dispensable	Deletion of ORF65: no effect in T cells and skin

DRG, dorsal root ganglia; EF-1α, elongation factor 1α; ERK, extracellular signal-regulated kinase; IDE, insulin-degrading enzyme; IE, immediate-early; IFN, interferon; ITIM, immunoreceptor tyrosine-based inhibition; JNK, c-JUN N-terminal kinase; PML, promyelocytic leukaemia protein; SIMs, SUMO-binding motifs; TGN, *trans*-Golgi network; VZV, varicella zoster virus.

*Function in cells and tissues was assessed by mutagenesis of the VZV genome using cosmids or bacterial artificial chromosomes (BACs) to delete or insert stop codons or introduce targeted changes in the coding sequence

‡Indicates differences among published observations about whether the gene is essential or dispensable, including variations in the mutations tested and/or experimental conditions used to assess growth requirement.