Cellular Stress Response to Varicella-Zoster Virus Infection of Human Skin Includes Highly Elevated Interleukin-6 Expression

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Background. The infectious cycle of varicella-zoster virus (VZV) after reactivation from the dorsal root ganglia includes replication and assembly of complete enveloped virions in the human skin to cause the characteristic herpes zoster (shingles). To pursue studies of innate immunity to VZV infection, we have adapted a fetal skin organ culture model to a human neonatal foreskin explant model.

Methods. To determine if increased transcription was accompanied by increased IL-6 expression, we quantitated the levels of IL-6 protein in the explant media at increasing intervals after infection. We found a statistically significant increase in IL-6 protein levels secreted into the media from VZV-infected skin explants as compared with mock-infected explants.

Results. Abundant expression of VZV IE62, gE, and gC was visualized by confocal microscopy while numerous enveloped virions were observed by electron microscopy in infected skin organ cultures. Microarray experiments demonstrated that the patterns of upregulated transcripts differed between VZV-infected cells and VZV-infected skin explants. One result stood out, namely a >30-fold elevated interleukin (IL)-6 level in the infected skin explant that was not present in the infected monolayer culture. The IL-6 results in the polymerase chain reaction (PCR) assay were reproduced by quantitative PCR testing with newly designed primers. To determine if increased transcription was accompanied by increased IL-6 expression, we quantitated the levels of IL-6 protein in the explant media at increasing intervals after infection. We found a statistically significant increase in IL-6 protein levels secreted into the media from VZV-infected skin explants as compared with mock-infected explants.

Conclusions. The cellular stress response to VZV infection in neonatal skin explants included highly elevated levels of IL-6 transcription and expression. This skin organ model could be adapted to other viruses with a skin tropism, such as herpes simplex virus.

Keywords. autophagy; cytokines; herpes virus; human skin; tocilizumab.

Varicella-zoster virus (VZV) is 1 of 9 human herpesviruses. VZV is a human pathogen that spreads to children as varicella or chicken pox and reemerges later in life as zoster or shingles [1, 2]. The virus is well adapted to its human host and infects most people in a given community [3]. The VZV genome is also the smallest among the human herpesviruses; it encodes 71 open reading frames, 3 of which are duplicated [4]. Of note, VZV lacks the herpes simplex virus 1 (HSV-1) ICP34.5 neurovirulence gene [5].

In earlier published studies, we have demonstrated that VZV infection induces endoplasmic reticulum (ER) stress, an unfolded protein response (UPR), and an autophagy response [6, 7]. In this manuscript, we describe our adaptation of a human fetal skin organ culture (SOC) model to further assess the innate immune responses to VZV infection [8]. We postulated that VZV infection of human skin explants would provide a more accurate representation of innate immunity than analysis solely of infected monolayer cultures. To assist in this investigation, we constructed 2 recombinant wild-type VZV strains that expressed either enhanced green fluorescent protein (eGFP) or monomeric red fluorescent protein (mRFP) as a fusion protein with VZV ORF11.

VZV pathogenesis has been extensively examined in human fetal skin xenografts inserted under the skin of a severe combined immunodeficient (SCID) mouse. Because of documented differences in VZV pathogenesis between cultured cells and skin xenografts, as defined in the SCID mouse model [9, 10], we examined the transcription of antiviral innate immune transcripts after VZV infection of both cultured monolayers and the newly devised SOC. There have been a number of reports that have examined the role cytokines play when released by activated T-cells to stimulate natural killer cells by IL-2, interferon (IFN)-α, or IFN-γ to result in lysis of VZV-infected cells [11, 12]. Other researchers have examined the effect VZV infection has on expression of immune modulators, such as MHC class II [13]. Through these studies, it has become clear that VZV infection can interfere with cellular responses to IFN and NF-kB transcription of inflammatory cytokine genes [14, 15].

In the current investigation, 1 result stood out in particular, namely elevated IL-6 expression in human skin. This result was of great interest to us because recent evidence points to IL-6 as a keystone immunomodulatory cytokine in both healthy...
and diseased tissues, including a related report about cultured human retinal cells [16].

METHODS

Viruses and Cells
VZV-rpOka is derived from a bacterial artificial chromosome (BAC) clone of the wild-type Oka virus (pOka; GenBank AB097933.1). Based on an earlier strategy for recombinant Marek’s Disease Virus (MDV) [17], we generated 2 VZV fluorescent recombinant strains with either eGFP (VZV-rpOka11G) or mRFP (VZV-rpOka11R) fused to the C-terminus of VZV ORF11 (Supplementary Figure 1). Prior studies in HSV-1 and MDV have shown that tagging the N- or C-terminus of the ORF11 homolog (VP13/14; UL47) gene did not affect expression of pUL46 (ORF12 homolog) or pUL48 (ORF10 homolog). VZV-32 is a low-passage laboratory strain (GenBank DQ479961.1). MRC-5 human fibroblast cells (ATCC) were grown in 6-well tissue culture plates containing either 12-mm round or 22-mm square coverslips in Minimum Essential Medium (MEM; Gibco, Life Technologies) supplemented with 7% fetal bovine serum (FBS). When monolayers were nearly confluent, they were inoculated with trypsin-dispersed VZV-infected cells at a ratio of 1 infected cell to 8 uninfected cells; the titer of infectious centers for a 25-cm² monolayer with extensive cytopathic effect is \(10^6\) [18]. Plaque assays for measurement of cell-free VZV titers have also been described [18].

Poly(I.C) Treatment of MRC-5 Cells
Poly(I:C), a dsRNA mimic, a positive control for induction of innate immunity, was transfected into MRC-5 cells using Lipofectamine 2000 (LF). Briefly, 10 µL of LF was dissolved into 100 µL of MEM, and then 1 µg of Poly(I:C) was added. The resulting solution was then added dropwise to medium overlying the cells.

Culture of Explant Skin Samples
De-identified specimens from circumcision procedures were collected from the nursery of the University of Iowa Children’s Hospital. Each skin sample was divided into 6-mm round pieces with a biopsy punch and placed onto the mesh bottom of Costar Netwell (Corning) tissue culture inserts (74-mm polystyrene mesh) in a 12-well plate containing 1.4 mL of complete MEM supplemented with nystatin and ciprofloxin. A single skin sample yielded between 8 and 12 individual pieces, each piece weighing about 0.03 g. Subsequently the plate was incubated at 32°C in a humidified incubator with 5% CO₂. Medium was replaced every 48–72 hours. We carried out 9 independent explant procedures.

Infection of Explant Skin Samples
Explanted neonatal skin was inoculated with cell-associated VZV. Each skin piece was pierced to the depth of approximately 1 mm (through the basement membrane into the dermis) in multiple sites with a 25-gauge hypodermic needle, and then an aliquot (500,000 cells in 200 µL) of infected cells was injected onto the surface of the epidermis and allowed to penetrate into the holes created by piercing the skin surface. Infected skin explants were incubated for as long as 28 days before sectioning and/or DNA/RNA extraction. There were 2 sets of controls. For the mock-infected control cultures, the skin samples were pierced at multiple sites with a 25-gauge hypodermic needle, then incubated under the same conditions as the infected cultures. In a second set of controls, duplicate VZV-infected monolayers were exposed to ultraviolet (UV) irradiation to inactivate all infectivity; the lack of infectivity was confirmed by passage of 1 of the 2 irradiated cultures onto a monolayer of uninfected cells. Then the skin pieces were inoculated with trypsin-dispersed irradiated infected cells from the second culture, as described above.

Extraction of RNA and DNA From VZV-Infected Human Skin
DNA and total RNA were extracted from individual skin samples using the Tissue Microarray RNeasy Kit (Qiagen). The described procedure consisted of a phenol-chloroform extraction, with the upper aqueous fraction being further purified in a Qiagen RNA purification column with on-column DNase treatment. The trapped RNA was eluted into 100 µL of water. Polyadenylated RNA was converted to cDNA using anchored Oligo(dT) primers and the SuperScript III First-Strand Synthesis System for Real-time Polymerase Chain Reaction (RT-PCR; Invitrogen), with subsequent reaction with the RNaseH enzyme to remove hybridized RNA:DNA sequences to yield approximately 20 ng of cDNA in 150 µL of water. The DNA fraction from the phenol-chloroform fractionation was then purified using the DNeasy kit (Qiagen) into 400 µL of elution buffer with the usual yield of 40 ng/µL. RNA and DNA extraction from cultured cells was described previously [6].

Polymerase Chain Reactions
The entire cDNA sample from 1 well of cells (or 1 skin explant) was mixed into 1 mL of IX diluted Power SYBR Green Master Mix (ABI) and split into all wells of a Qiagen RT2-Profiler antiviral response PCR array (PAHS-122ZA, Qiagen) with a multichannel pipettor (25 µL per well). For individual quantitative PCR (qPCR) measurements, 1 µL of DNA or cDNA was added to 25 µL of PCR reaction mixture containing specific primers (Supplementary Table 1). The measurements were carried out using a QuantStudio Flex 7 real-time PCR instrument (ABI). The resulting PCR results were processed using QuantStudio software (ABI). \(C_\text{r} \) values of each measurement were normalized to an average of 16.0 for housekeeping genes (wells H1–H5 of the antiviral response array) to form \(\Delta C_\text{r} \) values. Subsequent \(\Delta\Delta C_\text{r} \) values were calculated based on differences between measurements of VZV-infected \(\Delta C_\text{r} \) values or Poly(I:C)-treated \(\Delta C_\text{r} \) values and the uninfected \(\Delta C_\text{r} \) values or differences between VZV-infected skin values and uninfected skin values. Uncertainties were estimated based on previous qPCR measurements.
Samples of infected cells and skin sections were prepared for confocal microscopy by methods described previously [19]. Following preparation, the samples were viewed on a Zeiss 710 confocal laser scanning microscope. Murine monoclonal antibodies from the C. Grose laboratory included the following reagents: MAb 3B3 against VZV gE, MAb 5C6 against VZV IE62, and MAb 233 against VZV gC. We included immunohistochemical assays with isotype control antibodies for these anti-VZV antibodies in an earlier publication [20]. The In Situ Cell Death Detection (TUNEL) kit with fluorophores was purchased from Roche; slides were viewed with a confocal microscope. Methods for embedding and ultramicroscopy have been described elsewhere [19]. For the enumeration and comparison of viral particles under 3 conditions (VZV-infected SOC, human varicella skin biopsy, and VZV-infected monolayer), at least 50 particles were counted at each of 2 randomly selected locations on a TEM, for a total count of at least 100 particles. Viral particles were classified independently by 2 observers. The varicella skin biopsy was collected from a child with chickenpox on day 3 of illness; the VZV-infected cultures were processed for TEM when the cytopathic effect covered approximately 75% of the monolayer.

IL-6 Enzyme-Linked Immunosorbent Assay
The human IL-6 enzyme-linked immunosorbent assay kit was purchased from R&D Systems. Media samples were obtained at the University of Iowa, coded, and frozen, before being transported to the Mayo Clinic by same-day courier. The IL-6 results were analyzed, and Student t tests were performed to assess statistical significance.

Ethics Statement
De-identified foreskin samples were obtained from the nurseries of the University of Iowa Hospital. The project was reviewed by the University of Iowa Institutional Review Board, which considered the use of this human skin tissue to be exempt.

RESULTS
Implementation of Skin Organ Culture Model Using Human Foreskin Explants
The data below were selected from a total of 9 independent explant procedures from 9 different foreskin specimens. A photograph of the SOC model and a cartoon to illustrate the mode of infection are shown in Figure 1A. VZV rpOka showed a strong tropism for the cells in the epidermis of human skin with limited infection of dermal fibroblasts (Figure 1B). Results were measured when qPCR assays were performed on 6 independent skin samples, selected from at least 3 separate experiments (Figure 1C). Further, examination of the skin samples by confocal microscopy facilitated the detection of areas that were highly infected within every sample. There was similarity in degree of infectious foci between 14 and 28 dpi. We visualized the expression of 3 representative VZV proteins in the infected foci, including VZV gE, gC, and IE62 (Figure 1D, parts 1–9). VZV IE62 is the HSV ICP4 homolog, VZV gE is an abundantly produced gamma-1 protein, and VZV gC is a true late protein [21]. Control uninfected experiments are included in Figure 1D, parts 10–12; part 12 shows minimal TUNEL staining at 28 days, indicating that these explants can tolerate 28 days in culture without increased cell death.

Enumeration of Complete Virions After VZV Infection of Human Skin
VZV is renowned for producing mainly aberrant viral particles during assembly in infected monolayer cultures [22]. While examining VZV-infected human skin in the SOC samples, we postulated that there should be a greater number of complete virions in infected skin than was previously found in VZV-infected cell monolayers, if these infected explants authentically represented virus infection in human skin (Figure 2A–C). To this end, we quantitated the percentage of complete particles vs L-particles in VZV-infected human skin by counting particles in TEM images (Figure 2D). The same quantitation was applied to a TEM of a biopsy section from a chicken pox skin lesion [23]. We found almost 3 times more complete viral particles in both VZV-infected SOC and a chicken pox vesicle biopsy, as compared with VZV-infected cell monolayers (Figure 2D).

Comparison of Transcription in VZV-Infected Human Skin With Either Poly(I:C)-Treated Cells or VZV-Infected Cell Monolayers
Based on the data in the preceding “Results,” we concluded that the SOC model of VZV infection had many attributes of an authentic VZV infection of the human skin by 14 dpi. To compare innate immune transcription in response to VZV infection in monolayer cultures and in human skin explants, we employed a PCR array manufactured by Qiagen. This array consisted of 96 wells, with 12 wells dedicated to housekeeping transcripts and quality control transcripts. Each well contained primers bound to a specific transcript. The majority of the 84 wells were specific to innate immune transcripts that can be grouped by function (Supplementary Table 2): secreted cytokines including class I interferons, interleukins, and CC and CXC motif cytokines (20 wells); pathogen-associated molecular patterns (PAMPs) including Toll-like receptor (TLR) transcripts (16 wells); apoptosis-related, primarily caspase, transcripts (10 wells); signaling kinases (10 wells); interferon-stimulated transcripts (9 wells); NFκB components (10 wells); inflammatory transcripts (8 wells); and others (3 wells). Five samples of cDNA were added to 5 different arrays: untreated and uninfected MRC-5 cells as baseline control samples, Poly(I:C)-treated MRC-5 cells, VZV-infected MRC-5 cells at 72 hpi, and finally both uninfected human skin after 14 days of incubation and VZV-rpOka11R-infected human skin at 14 dpi.

After treatment with poly(I:C), the majority of transcripts (54 of 84) in the PCR array were upregulated, with IFN-β being the most upregulated (Figure 3A). Many other transcripts (cytokine
and interferon stimulated) were also upregulated, presumably partly in reaction to the increased level of IFN-β in the medium. In VZV-infected cells, 18 transcripts were upregulated, most notably tumor necrosis factor–alpha (TNF-α). Downregulation was seen for another 20 transcripts (Figure 3B; Supplementary Table 3). By comparison, many more innate immune transcripts (40 transcripts) were upregulated in uninfected human skin after 14 days of incubation than uninfected MRC-5 cell monolayers after a similar incubation period, whereas the MAP kinases were among 19 transcripts that were downregulated (Figure 3C; Supplementary Table 3).

Finally, infection of human skin explants upregulated relatively few transcripts (Figure 3D; Supplementary Table 3). These included the following 14: IL-6, IFNB1, TLR7, CXCL8, IFNA2, CXCL9, CXCL10, CCL3, IRAK1, SPP1, TNF, CXCL11, CTSL, and CCL5. When compared with the results in VZV-infected cell monolayers, only the following transcripts were upregulated in both substrates: TNF, TLR7, CCL3, SPP1, INFβ1,
and CXCL8. When transcripts upregulated in uninfected skin explants were removed from the list, only TLR7 and IFNB1 remained. In particular, we point out that IL-6 was the single most elevated transcript in the profile of infected human skin (Figure 3D). Among the cytokines, IL-6 was the only cytokine upregulated to the same degree (~32-fold) by VZV skin infection as by Poly(I:C), yet IL-6 was not induced in infected cell monolayers. Four transcripts failed to be amplified in the PCR array: TLR8 in Poly(I:C)-treated cells, VZV-infected cells, and VZV-infected human skin; and MEFV in VZV-infected human skin. In Figure 3 and Supplementary Table 3, these values are set to 0 with no error bars. Repeat experiments by qPCR with individual primers for TRL8 and MEFV are shown in Figure 4, C and D.

**Confirmation of IL-6 Transcription in Microarray by Independent qPCR Assay**

After reviewing the data in Figure 3, we decided to limit further assessment to the cytokines. Values are shown in Figure 4A for all cytokine transcripts with transcription above 1.5 from the PCR array; the color refers to the sample: Poly(I:C)-treated (red), VZV-infected cells (green), and infected human skin (blue), rather than a functional grouping, as shown in Figure 3. We performed independent qPCR measurements using newly designed primers to confirm the PCR array values (Supplementary Table 1). We further narrowed our analysis to 4 sets of transcripts, including IFN-α, IFN-β, TNF-α, and IL-6 in Poly(I:C)-treated MRC-5 cells, VZV-infected cells at 72 hpi, and finally VZV-rpOka11R-infected human skin. The new qPCR assays were designed against regions of the gene transcript that were different from the primer targets in the commercial microarray. Overall, there was general agreement between results in the microarray and the independent measurements with specific individual primers (Figure 4B). Even an almost identical upregulation of IL-6 transcription in Poly(I:C)-treated cells and infected human skin cells was confirmed. Because of the importance of the IL-6 assay, a Student t test was carried out on the cumulative results from 3 independent qPCR assays. The difference between the IL-6 values of either the Poly(I:C)-treated or the VZV-infected skin, when compared with the VZV-infected monolayer, was significant (P < .001). Subsequent studies were confined to IL-6.

**Quantitation of IL-6 Protein Levels in VZV-Infected SOC**

After documenting increased IL-6 transcription in VZV-infected SOC by 2 independent assays, we hypothesized that an upregulation in transcription would be accompanied by a subsequent elevated level of IL-6 protein expression. Studies were carried out in 4 separate experiments, with skin specimens from 4 different donors. Media samples that surrounded the

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**Figure 2.** Higher number of complete enveloped viral particles in varicella-zoster virus (VZV)–infected skin explants. Cryosections of VZV-infected human skin were processed for TEM and viewed at high magnification in a JEOL 1230 microscope. A, VZV-infected epidermis exhibited numerous viral particles in the cytoplasm of both basal and suprabasal keratinocytes. B, Higher magnification of the boxed area in (A): complete viral particle (solid arrow) and incomplete viral particle (L-particles; dashed arrow). Note that L-particles often have a larger diameter than complete particles. C, Higher magnification of a single cytoplasmic vesicle containing viral particles that are distinct enough to classify as complete or L-particles. D, Comparison of the percentage of complete virions vs aberrant particles in VZV-infected cell monolayers (prior work), VZV-rpOka11R-infected human skin explants (present work) and a typical chicken pox vesicle (prior work). Criteria for classification of viral particles and their enumeration are described in the “Methods.” Abbreviation: Nuc, nucleus.
SOC, either mock-infected or infected with VZV, were saved and analyzed for the presence of IL-6 protein. We found a statistically significant increase in IL-6 protein levels secreted into the media from VZV-infected SOC at 2 weeks post-infection as compared with the mock-infected SOC from the same time point ($P = .0006$) (Figure 5A). As an additional control experiment for mock infection, we inactivated our inoculum of VZV-infected cells by exposure to UV irradiation before injection into the skin explants. The IL-6 expression in the latter cultures was similar to mock-infected cultures. Further, both infected and uninfected cell monolayers showed little expression of IL-6 in the media (levels were below the lowest point of the standard curve for this assay). Therefore, the difference between IL-6 levels in media around SOC infected for 2 weeks and media overlying monolayers infected for 3 days (extensive cytopathic effect) was highly significant ($P \leq .0001$) (Figure 5B). Because IL-6 has a half-life <24 hours and the medium was changed every 2–3 days, the higher levels in infected SOC cultures did not represent accumulation of IL-6.

**DISCUSSION**

The SCID mouse with human skin xenographs has been an informative model for VZV infection of skin [10]. The neonatal SOC system is an alternative model with some advantages, for example, ease and rapidity of increasing the number of skin samples per experiment. Virus is introduced in both models by injection into the skin near the dermal/epidermal junction. Thus, neither model duplicates infection of skin via the viremia found in humans with varicella [1]. However, the SOC model more closely duplicates herpes zoster, where virus directly enters the epidermis from distal sensory nerve fibers.
Documentation of a marked increase in IL-6 transcription and expression in VZV-infected skin adds to our knowledge about innate immunity and VZV pathogenesis in humans (Supplementary Figure 2). IL-6 was called B-cell stimulatory factor (BSF-2) or interferon beta-2 (IFNB2) when first discovered. IL-6 has a multiplicity of functions that involve both pro-inflammatory and anti-inflammatory effects related to innate immunity within different microenvironments [24]. IL-6 is secreted by T cells, macrophages, and many stromal cells and has a half-life less than 24 hours. The source of most IL-6 transcription in our human skin experiments may be the infected keratinocyte. Prior studies have shown an induction of IL-6 transcription in wounded human keratinocytes [25]. Presumably, VZV infection would simulate a wound lesion in these cells. We propose that lymphocytes residing within the skin were not a primary source of IL-6, because IL-6 production persisted in the SOC through 28 days, a time when human lymphocyte subpopulations would be declining during their relatively short half-life [26]. This result may also clarify some IL-6 results from human subjects with herpes zoster. For example, 2 patients with herpes zoster/postherpetic neuralgia (HZ/PHN) had high IL-6 transcription levels in affected skin [27]. In another study, 6 patients with herpes zoster had anti-IL-6 antibodies [28]. In a third study, elevated levels of IL-6 protein were found in the cerebrospinal fluid of patients with central nervous system disease secondary to VZV vasculopathy [29].

Studies with IL-6 have been carried out with several RNA viruses. After a literature review, we found that our VZV IL-6 data were most similar to a description of elevated IL-6 following hepatitis B infection [30]. During hepatitis B infection, dysregulated production of the viral surface proteins leads to their retention in the ER with subsequent ER stress. When the latter investigators transfected human liver cells, they documented an upregulation of IL-6 at both the mRNA and protein levels. These authors and others have observed that IL-6 expression can be regulated by a CCAAT-enhancer-binding protein homologous protein (CHOP) pathway. CHOP is a 29-kDa protein that is a member of the C/EBP family of transcription factors; CHOP has also been called growth arrest and DNA damage protein 153 (GADD153). CHOP production is also augmented by
increased IL-6 [31]. We have previously documented that CHOP was upregulated in VZV-infected monolayers in concert with the appearance of the spliced form of XBP1; spliced XBP1 is another distinctive signal of ER stress [6]. These dual concordant results suggested that as VZV infection progressed, the conditions of ER stress in the cells became extreme.

Crosstalk between IL-6 and Toll-like receptor signaling networks has been documented [32]. A recent relevant paper describes a previously unrecognized link between IL-6 and enhanced autophagy via the STAT3-MIR155-3p-CREBF-CREB3-ATG5 pathway [33]. In another study, VZV infection led to increased phosphorylation of STAT3, evidence for the first step in the IL-6-STAT3 autophagy pathway [34]. When these VZV data are considered together, we conclude that there is increasing evidence suggesting elevated levels of IL-6 as a marker of an enhanced cellular stress response of human skin to VZV infection.

Supplementary Data
Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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References
1. Weller TH. Varicella and herpes zoster. Changing concepts of the natural history, control, and importance of a not-so-benign virus. N Engl J Med 1983; 309:1434–40.
2. Gershon AA, Breuer J, Cohen II, et al. Varicella zoster virus infection. Nat Rev Dis Primers 2015; 1:15016.
3. Hope-Simpson RE. The nature of herpes zoster: a long-term study and a new hypothesis. Proc R Soc Med 1965; 58:9–20.
4. Davison AJ, Scott JE. The complete DNA sequence of varicella-zoster virus. J Gen Virol 1986; 67(Pt 9):1759–816.
5. Orvedahl A, Alexander D, Tallóczy Z, et al. HSV-1 ICP34.5 confers neurovirulence by targeting the Bcl-2 family. Cell Host Microbe 2007; 12:3–15.
6. Carpenter JE, Jackson W, Benetti L, Grose C. Autophagosome formation during varicella-zoster virus infection following endoplasmic reticulum stress and the unfolded protein response. J Virol 2011; 85:9414–24.
7. Carpenter JE, Grose C. Varicella-zoster virus glycoprotein expression differentially induces the unfolded protein response in infected cells. Front Microbiol 2014; 5:322.
8. Taylor SL, Moffat JE. Replication of varicella-zoster virus in human skin organ culture. J Virol 2005; 79:11501–6.
9. Moffatt JE, Zerbini L, Kinchington PR, et al. Attenuation of the vaccine Oka strain of varicella-zoster virus and role of glycoprotein C in alpha-herpesvirus virulence demonstrated in the SCID-hu mouse. J Virol 1998; 72:965–74.
10. Arvin AM. Investigations of the pathogenesis of varicella zoster virus infection in the SCIDhu mouse model. Herpes 2006; 13:75–80.
11. Mizutani K, Ito M, Kamiya H, Sakurai M. Inhibition of varicella-zoster virus glycoprotein expression by peripheral blood mononuclear cells. Microbiol Immunol 1995; 39:217–20.
12. Torigo S, Ishii T, Kamiya H. IL-12, IFN-gamma, and TNF-alpha released from mononuclear cells inhibit the spread of varicella-zoster virus at an early stage of varicella. Microbiol Immunol 2000; 44:1027–31.
13. Abendroth A, Slobedman B, Lee E, et al. Modulation of major histocompatibility class II protein expression by varicella-zoster virus. J Virol 2000; 74:1900–7.
14. Sen N, Sommer M, Che X, et al. Varicella-zoster virus immediate-early protein 62 blocks interferon regulatory factor 3 (IRF3) phosphorylation at key serine

Figure 5. Measurement of interleukin (IL)-6 protein levels. Human IL-6 protein levels in the cell-free media from skin organ model (SOC) samples were measured by enzyme-linked immunosorbent assay. SOC media were changed twice weekly. The groups included uninfected and infected skin samples in triplicate, which were incubated for increasing time periods, from day 4 to day 28. Another group called monolayers consisted of media samples from 3 uninfected and 3 infected human cell monolayers, which were incubated for 3 days. Another group consisted of SOC that was infected with ultraviolet-inactivated varicella-zoster virus (VZV) and media harvested at day 14. The number of asterisks indicates the degree of statistical significance. A, Comparison of media from SOC groups with media from uninfected cell monolayers. B, Comparison of media from VZV-infected SOC at 2 weeks (when foci of infectivity were easily found) with media from VZV-infected monolayers at 3 days (when cytopathic effect was evident).
residues: a novel mechanism of IRF3 inhibition among herpesviruses. J Virol 2010; 84:9240–53.
15. Vandevenne P, Lebrun M, El Mjiyad N, et al. The varicella-zoster virus ORF47 kinase interferes with host innate immune response by inhibiting the activation of IRF3. PLoS One 2011; 6:e16870.
16. Graybill C, Claypool DJ, Brinton JT, et al. Cytokines produced in response to varicella-zoster virus infection of ARPE-19 cells stimulate lymphocyte chemotaxis. J Infect Dis 2017; 216:1038–47.
17. Jarosinski KW, Arndt S, Kaufer BB, Osterrieder N. Fluorescently tagged pUL47 of Marek’s disease virus reveals differential tissue expression of the tegument protein in vivo. J Virol 2012; 86:2428–36.
18. Grose C, Brunel PA. Varicella-zoster virus: isolation and propagation in human melanoma cells at 36 and 32 degrees C. Infect Immun 1978; 19:199–203.
19. Carpenter JE, Hutchinson JA, Jackson W, Grose C. Egress of light particles among filopodia on the surface of varicella-zoster virus-infected cells. J Virol 2008; 82:2821–35.
20. Buckingham EM, Foley MA, Grose C, et al. Identification of herpes zoster-associated temporal arteritis among cases of giant cell arteritis. Am J Ophthalmol 2018; 187:51–60.
21. Storlie J, Carpenter JE, Jackson W, Grose C. Discordant varicella-zoster virus glycoprotein C expression and localization between cultured cells and human skin vesicles. Virology 2008; 382:171–81.
22. Carpenter JE, Henderson EP, Grose C. Enumeration of an extremely high particle-to-PFU ratio for varicella-zoster virus. J Virol 2009; 83:6917–21.
23. Grose C, Varicella zoster virus: pathogenesis of the human diseases, the virus and viral replication, and the major viral glycoproteins and proteins. In: Hyman RW, ed. Natural History of Varicella-Zoster Virus. Boca Raton, FL: CRC Press, Inc. 1987:1–65.
24. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. Nat Immunol 2015; 16:448–57.
25. Sugawara T, Gallucci RM, Simeonova PP, Luster MI. Regulation and role of interleukin 6 in wounded human epithelial keratinocytes. Cytokine 2001; 15:328–36.
26. Hellerstein M, Hanley MB, Cesar D, et al. Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. Nat Med 1999; 5:83–9.
27. Uçeyler N, Valer M, Kalke W, et al. Local and systemic cytokine expression in patients with postherpetic neuralgia. PLoS One 2014; 9:e105269.
28. Bayat A, Burbelo PD, Browne SK, et al. Anti-cytokine autoantibodies in postherpetic neuralgia. J Transl Med 2015; 13:333.
29. Jones D, Alvarez E, Selva S, et al. Proinflammatory cytokines and matrix metalloproteinases in CSF of patients with VZV vasculopathy. Neuroi Neuroinflamm 2016; 3:e246.
30. Li YX, Ren YL, Fu HJ, et al. Hepatitis B virus middle protein enhances IL-6 production via p38 MAPK/NF-κB pathways in an ER stress-dependent manner. PLoS One 2016; 11:e0159089.
31. Zhang K, Shen X, Wu J, et al. Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. Cell 2006; 124:587–98.
32. Mansell A, Jenkins BJ. Dangerous liaisons between interleukin-6 cytokine and Toll-like receptor families: a potent combination in inflammation and cancer. Cytokine Growth Factor Rev 2013; 24:249–56.
33. Xue H, Yuan G, Guo X, et al. A novel tumor-promoting mechanism of IL6 and the therapeutic efficacy of tocilizumab: hypoxia-induced IL6 is a potent autophagy initiator in glioblastoma via the p-STAT3-MIR155-3p-CREBRF pathway. Autophagy 2016; 12:1129–52.
34. Sen N, Che X, Rajamani J, et al. Signal transducer and activator of transcription 3 (STAT3) and survivin induction by varicella-zoster virus promote replication and skin pathogenesis. Proc Natl Acad Sci U S A 2012; 109:600–5.
35. Jarosinski KW, Vautherot JF. Differential expression of Marek’s disease virus requires US2, the UL13 protein kinase, and gC. J Virol 2007; 81:13200–8.
36. Donnelly M, Elliott G. Nuclear localization and shuttling of herpes simplex virus tegument protein VP16-UL49 gene locus. Virology 2015; 484:213–26.
37. Kinchington PR, Houglund JR, Arvin AM, et al. The varicella-zoster virus immediate-early protein IE62 is a major component of virus particles. J Virol 1992; 66:359–66.
38. Tischer BK, Kaufer BB, Sommer M, et al. A self-excisable infectious bacterial artifical chromosome clone of varicella-zoster virus allows analysis of the essential tegument protein encoded by ORF9. J Virol 2007; 81:13200–8.
39. Jarosinski KW, Margulis NG, Kamil JP, et al. Horizontal transmission of Marek’s disease virus requires US2, the UL13 protein kinase, and gC. J Virol 2007; 81:10575–87.
40. Osterrieder N, Kamil JP, Schumacher D, et al. Marek’s disease virus: from miasma to model. Nat Rev Microbiol 2006; 4:283–94.