Tropism of Varicella-Zoster Virus for Human CD4⁺ and CD8⁺ T Lymphocytes and Epidermal Cells in SCID-hu Mice

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To investigate the cell tropism and pathogenicity of varicella-zoster virus (VZV) strains, we analyzed VZV replication by using SCID-hu mice that carry human fetal thymus/liver implants under the kidney capsule or as subcutaneous fetal skin implants. MRC-5 cells infected with wild-type VZV or the Oka strain, used in the live attenuated varicella vaccine, were injected into the implants. The implants were surgically removed 2, 7, 14, and 21 days postinfection. The VZV titer from infected thymus/liver implants peaked on day 7 for the wild-type strain and on day 14 for the Oka strain. Histological analysis showed necrotic areas characterized by thymocyte depletion and fibrosis. VZV protein synthesis was detectable by immunohistochemical staining in the necrotic areas and in distant regions that did not show cytopathic changes, and VZV DNA was detected by *in situ* hybridization in the same distribution. Fluorescence-activated cell sorting analysis of thymocytes harvested at day 7 postinfection showed that VZV proteins were expressed in CD4⁺, CD8⁺, and CD4⁺ CD8⁺ T cells; VZV was cultured from each T-cell subpopulation. The Oka strain had tropism for human cell types similar to that of wild-type VZV. T lymphocytes released infectious VZV, which is a novel and important observation about the replication of this otherwise highly cell associated virus. VZV-infected skin implants exhibited microscopic epidermal lesions that were indistinguishable histologically from the characteristic lesions of varicella. These experiments demonstrate a unique tropism of VZV for human T lymphocytes, explaining its capacity to cause viremia in natural disease, and demonstrate the value of the SCID-hu model for studies of VZV pathogenesis.

Primary infection with varicella-zoster virus (VZV) causes chickenpox (varicella). The virus establishes latency in dorsal root ganglia and can reactivate years later to cause shingles (herpes zoster). Cell-associated viremia occurs during the incubation period of varicella and leads to the eruption of the widespread vesiculopapular exanthem which characterizes this disease and distinguishes it from infection with herpes simplex virus, which is the prototype of the alphaherpesviruses (3, 7, 30).

The lack of a small animal model which mimics human disease is a major obstacle to the study of events in VZV pathogenesis. In contrast to HSV, VZV is not infectious for mice; guinea pigs are susceptible to transient infection with VZV but show few or no signs of disease (20–22). There are no systems available for investigating VZV replication in epidermal cells. The advantage of the SCID-hu mouse system is that these immunodeficient mice can be used to support grafts of various human tissues and the grafted tissues will differentiate to contain the usual human cell populations (17). Human tissue implants in SCID-hu mice permit studies of the replication of other human viruses that are highly species specific, including human immunodeficiency virus (23, 27) and human cytomegalovirus (18).

We have developed a model for investigating VZV replication *in vivo*, using SCID-hu mice implanted with human fetal thymus/liver (thy/liv) conjoint tissue or with fetal skin. This SCID-hu model has provided the first opportunity to examine VZV tropism for cell types that constitute specific human tissues *in vivo* and to compare VZV strains for differences in pathogenic potential. Comparisons were done with wild-type and Oka strains because the Oka strain, which has been passaged in guinea pig embryo cells, is being used to produce a live

attenuated varicella vaccine (29). Experiments in the SCID-hu model established the tropism of VZV for human T lymphocytes and permitted the examination of VZV pathogenesis in skin, which is a critical site of viral replication in natural infection. In the thy/liv implants, the virus infected T cells in the lymphoid lobes while sparing the thymic epithelium. VZV infection caused epidermal changes in skin implants that were indistinguishable from biopsies of natural varicella lesions (7). The SCID-hu model provides a new system for analyzing the relationships between viral gene expression and human cell tropism, using naturally arising VZV variants and recombinant VZV strains that carry directed mutations.

MATERIALS AND METHODS

SCID-hu mice. Male homozygous C.B-17 *scid/scid* mice were bred and maintained at SyStemix, Inc. At 8 weeks of age, coimplants of human fetal thymus and liver tissue from 18- to 23-week fetuses were introduced under the kidney capsule as a conjoint implant, using an 18-gauge trocar (24). Skin was introduced subcutaneously as full-thickness dermal grafts. Human fetal tissues were obtained with informed consent according to federal and state regulations and were screened for human immunodeficiency virus. The general care of the experimental animals used for this study was done in accordance with National Institutes of Health guidelines for laboratory animals and in compliance with the Animal Welfare Act (Public Law 94-279). The specific project was reviewed and approved by the Stanford University Administrative Panel on Laboratory Animal Care.

Viral strains and culture conditions. A fresh clinical VZV isolate, designated strain Schenke, and VZV Oka strain (American Type Culture Collection) were passed twice in human foreskin fibroblasts and twice in MRC-5 human lung fibroblasts and stored at -70°C in tissue culture medium (TCM) (minimal essential medium; Mediatech, Washington, D.C.) supplemented with 2 mM L-glutamine (Gibco, Gaithersburg, Md.), 50 IU of penicillin and 50 μg of streptomycin (Pen/Strep; ICN Biomedicals, Inc., Costa Mesa, Calif.), 0.5 μg of amphotericin B (Fungizone; Flow Laboratories, McLean, Va.) plus 10% dimethyl sulfoxide. Animal inoculations were done with VZV-infected MRC-5 cells that were maintained in TCM with 10% fetal calf serum (FCS; Tissue Culture Biologicals, Tulare, Calif.) and used at 3 to 4+ cytopathic effect (CPE). The VZV-containing monolayer was trypsinized, and the cells were counted, centrifuged, resuspended in TCM, and briefly stored on ice until they were injected into the SCID-hu mouse implants. Mock-infected implants were injected with an equal

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number of uninfected MRC-5 cells prepared in the same manner. The virus inoculum was determined by plaque titration on Vero cell monolayers for each experiment. Vero cells were passaged twice weekly and maintained in TCM with 5% FCS at 37°C in humidified 5% CO₂. Unless otherwise stated, all chemicals used were obtained from Sigma Chemical Co., St. Louis, Mo.

Infection of thy/liv implants. Experiments in SCID-hu mice with thy/liv implants were done 1 to 6 months after implantation. The mice were anesthetized intraperitoneally with a solution of 5% ketamine (Aveco Co., Inc., Fort Dodge, Iowa) and 2.5% xylazine (LypheMed, Inc., Rosemont, Ill.) (wt/vol in phosphate-buffered saline [PBS; 140 mM NaCl, 2.7 mM KCl, 15 mM Na₂HPO₄, 1.5 mM KH₂PO₄, pH 7.6]), and the left kidney was exposed surgically. The implant, which appeared as a 30- to 100-mm³ pale tissue, was inoculated with approximately 20 to 50 µl of a suspension of VZV-infected cells by using a 27-gauge needle. The peritoneal incision was sutured, the skin was stapled closed, and the animals were observed daily for abnormal behavior or illness. At 2, 7, 14, and 21 days postinoculation, the implants were removed. Half of each tissue block was fixed in 10% formalin; the other half was compressed and disrupted between ground glass slides. The cell suspension was filtered through a sterile nylon mesh to remove large debris, and the released cells were counted, washed, and used for fluorescence-activated cell sorting (FACS) analysis and virus titration.

Infection of skin implants. Three to five weeks after implantation of human fetal skin, the mice were anesthetized, an 8-mm dorsal, midsagittal incision was made, and the bilateral skin implants were exposed for inoculation with wild-type VZV (clinical strain Schenke). One graft was inoculated by scraping the surface with a scalpel and covering it with 1 drop of the inoculum from a 27-gauge needle; 1 to 10 µl of the inoculum was injected into the other graft by using a 27-gauge needle. Control implants were inoculated with an equal number of uninfected MRC-5 cells. The skin was then stapled closed to cover each implant. At 7, 14, and 21 days postinoculation, the implants were dissected from the murine skin and divided; one half was fixed in 10% formalin, and the other half was frozen in PBS at -20°C.

Infectious focus assay. Cells from thy/liv implants or small pieces of skin implants were serially diluted 10-fold in TCM with 5% FCS. A 0.1-ml cell suspension, mixed with 1.5×10^5 Vero cells in 0.9 ml of TCM, was added to 24-well dishes in triplicate. The dishes were incubated for 6 days at 37°C in 5% CO₂; 1.0 ml of fresh TCM with 5% FCS was added on day 3. Following aspiration of the supernatant, the wells were flooded with crystal violet stain (5% ethanol, 5% formaldehyde, and 0.13% crystal violet in PBS) for 2 to 5 min. The stain was aspirated, the wells were air dried, and the plaques were counted in an inverted light microscope (magnification, $\times 40$). The number of infectious foci per implant and the titer of the inoculum were calculated. The difference in virus titer was analyzed for statistical significance, using the Student *t* test.

A modification of the infectious focus assay was performed with 3.0-µm-pore-size transwells (Costar Corp., Cambridge, Mass.). Cell suspensions of infected and uninfected thy/liv implants were obtained 7 days after inoculation and added to the transwell chamber at concentrations of 10^5 cells in 0.1 ml of TCM. The transwells were placed in 24-well tissue culture plates seeded with Vero cell monolayers in 1.0 ml of TCM and incubated for 6 days; the monolayers were stained with crystal violet and examined for characteristic VZV plaques by light microscopy.

FACS analysis and cell separation for infectious focus assay. Aliquots of approximately 10^6 lymphocytes obtained from infected and uninfected thy/liv implants were washed in PBS with 2% FCS, resuspended in 100 µl of PBS with 2% FCS in a 96-well V-bottom microtiter dish, and incubated on ice with VZV-immune or nonimmune polyclonal immunoglobulin G diluted 1:40 in PBS with 2% FCS for 45 min. The immunoglobulin G fractions of these antibodies were isolated from the sera of immune and nonimmune human donors. The cell samples were washed by layering 100 µl of FCS under the cells, centrifuging the plates, and aspirating the supernatant. The cells were resuspended in 100 µl PBS with 2% FCS and incubated on ice with mouse monoclonal anti-CD4-phycoerythrin (Becton Dickinson, Mountain View, Calif.) and anti-CD8-tricolor (CalTag Laboratories, South San Francisco, Calif.) conjugates (both diluted 1:50) and with goat anti-human antibody-fluorescein isothiocyanate-conjugated F(ab')₂ fragments (diluted 1:100; CalTag) for 45 min. The cells were washed again and resuspended in PBS with 2% FCS and 1% paraformaldehyde. Cell suspensions were analyzed with a Becton Dickinson FACScan apparatus.

To separate T-cell subpopulations for the infectious focus assay, approximately 10^6 lymphocytes from thy/liv implants were treated with anti-CD8-fluorescein isothiocyanate (Becton Dickinson) and anti-CD4-phycoerythrin antibody conjugates (both diluted 1:50) on ice for 45 min. The cells were washed, resuspended in 0.5 ml of PBS with 2% FCS, and sorted into CD4⁺, CD4⁺CD8⁺, and CD8⁺ subpopulations with a Becton Dickinson FACScan. Each sample was centrifuged, resuspended in 0.5 ml of PBS with 2% FCS, and titered on Vero cells by using the infectious focus assay.

Histology and immunohistochemistry. Thy/liv and skin implants were fixed in 10% formalin overnight, embedded in paraffin, cut into 3-µm sections, and stained with hematoxylin and eosin (H&E). Unstained sections were deparaffinated in xylene for 3 min and rehydrated in graded ethanol before use. For immunohistochemistry, tissue sections were treated with a polyclonal human anti-VZV serum for 30 min; the polyclonal serum was obtained from a patient with herpes zoster and diluted 1:500 in PBS with 2% FCS and 10 mM Na₂S₂O₈. Sections were then rinsed twice with PBS, and a secondary biotinylated goat

anti-human antibody (diluted 1:1,000 in PBS with 2% FCS and 10 mM Na₂S₂O₈) was added for 40 min, and the sections were rinsed again with PBS followed by Tris-buffered saline (TBS; 125 mM NaCl, 15 mM Tris, pH 8.0). Sections were then incubated in a streptavidin-alkaline phosphatase conjugate for 40 min, diluted 1:400 in TBS with 2% FCS and 10 mM Na₂S₂O₈ (Jackson ImmunoResearch Labs, Inc., West Grove, Pa.), and rinsed twice with TBS. The red color was produced by flooding the tissue sections with a fast red substrate mix (2% dimethylformamide, 0.1% fast red, 0.02% naphthol AS-MX phosphate, 100 mM Tris, pH 8.2) for 20 to 40 min. The slides were rinsed with water to stop the reaction, counterstained for 15 s with hematoxylin, air dried, and covered with Crystal/Mount (Biomedex Corp., Foster City, Calif.). After drying, the slides were coverslipped with Permount (Fisher Scientific, Fair Lawn, N.J.) and examined by light microscopy.

In situ hybridization. The VZV probe consisted of a 12.9-kb biotinylated plasmid, pVZV-C, that is a pBR322 vector carrying the *Hind*III fragment C of VZV genomic DNA (13). The probe was biotinylated by using a BioNick kit (Bethesda Research Laboratories, Gaithersburg, Md.) according to the manufacturer's instructions and purified by ethanol precipitation. A negative control probe consisting of pBR322 vector alone was prepared in the same manner and used at the same concentration as the VZV-specific probe. The sectioned and deparaffinated tissue was treated with 3 µg of proteinase K per ml for 30 min in a humidified chamber at 37°C and then rinsed with water. The hybridization solution (5× SSC [1× SSC is 0.15 M NaCl and 0.015 M sodium citrate], 1× Denhardt's solution, 100 µg of salmon sperm DNA per ml, 80 ng of VZV probe DNA, 40% formamide) was placed on the tissue section, which was coverslipped and denatured at 82°C for 10 min.

Following hybridization overnight in a humidified chamber at 37°C, the coverslip was removed with Triton buffer (50 mM Tris base, 0.5 mM NaCl, 0.1% Triton X-100, pH 7.5), the slide was rinsed with TBS, and the tissue was treated with streptavidin-alkaline phosphatase for 40 min, rinsed with TBS and then rinsed Tris-saline (100 mM Tris base, 100 mM NaCl, 50 mM MgCl₂, pH 9.5). The blue color was produced by adding an nitroblue tetrazolium-5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt (BCIP) reagent for 20 to 40 min (0.0035% nitroblue tetrazolium chloride and 0.0017% BCIP [Amresco, Solon, Ohio] in Tris-saline) and then rinsing the tissue with water. The tissue sections were counterstained with hematoxylin, coated with Crystal/Mount, and coverslipped with Permount.

RESULTS

Replication of wild-type VZV and Oka strains in thy/liv implants. The replication of wild-type VZV and Oka strains in thy/liv implants was measured over 21 days, using the infectious focus assay. Lymphocytes were recovered from infected and uninfected implants; the number of infected cells was determined by plaque titration on Vero cell monolayers. The number of VZV-infected MRC-5 cells in the inoculum was measured in the same assay. The total number of infectious foci is shown for each thy/liv implant (Fig. 1A); at least three implants were analyzed for each timepoint. Although there was some variation between implants, the levels of replication of wild-type and Oka strains were comparable except on day 14 postinfection. Comparison of the geometric means of the titers showed that wild-type VZV replicated quickly up to day 7 postinfection to a peak of 5.0×10^4 and decreased at day 14, with no infectious virus detectable by day 21 (Fig. 1B). The Oka strain grew somewhat more slowly, reaching a peak titer of 8.0×10^4 on day 14, which was significantly higher than the wild-type strain titer of 1.1×10^4 on day 14 (Student *t* test *P* = 0.04). The failure to recover infectious virus by day 21 from implants inoculated with the wild-type or Oka strain correlated with an extensive depletion of thymic CD4⁺ and CD8⁺ lymphocytes, as determined by histological and FACS analysis. No VZV was isolated from the uninfected control implants.

Histological analysis of thy/liv implants infected with VZV. The CPE and the distribution of viral protein expression and viral DNA in infected implants were demonstrated by histological and immunohistochemical analysis. H&E-stained sections of VZV-infected thy/liv implants revealed progressive CPE over the 21-day course of viral replication. On day 2 postinfection, very little evidence of CPE was seen in the lymphocytes or thymic epithelium. Macrophages had infiltrated the implants and engulfed cells which expressed VZV proteins, as shown by immunohistochemical staining; however, it was

FIG. 1. Replication of VZV in thy/liv implants. The number of infectious foci per implant was determined by titration of infected lymphocytes on Vero cell monolayers. The average titers of the inoculum of infected MRC-5 cells from three experiments were calculated to be $3.2 \times 10^3 \pm 1.1 \times 10^3$ (mean $\pm$ standard error) for the wild-type strain and $6.5 \times 10^3 \pm 2.9 \times 10^3$ for the Oka strain. The scattergraph (A) shows the total number of infectious foci for each thy/liv implant infected with wild-type (open squares) and Oka (closed circles) strains of VZV. Panel B shows the geometric means of the scatter data; the bars represent the standard error of the mean. Only positive samples were used to calculate the data shown for day 14. Dotted lines represent the level of detection of the assay.

not possible to determine whether the macrophages became infected (Fig. 2A). On day 7, the lymphoid lobes showed areas of fibrosis, interstitial hemorrhage, and lymphocyte depletion (Fig. 2B) that contrasted with the completely normal histologic appearance of uninfected implant tissue stained with H&E and the absence of any viral DNA signal or protein expression in these specimens (Fig. 2B'). Necrosis was pronounced in infected implants by day 14. In situ hybridization and immunohistochemical staining revealed extensive viral DNA and protein expression in the tissue sections (Fig. 2C and D). Viral protein expression was apparent in an even distribution throughout the lymphoid lobes of the implant, but trace amounts of VZV antigen were detected in cells composing the thymic

stroma or capsule structures. The in situ hybridization signal for viral DNA was strong but present only in areas where lymphocytes remained intact; clear regions without a VZV DNA signal were observed in the areas with the most severe necrosis. At day 21, the infected implants were involuted and shrunken; residual evidence of viral protein expression and viral DNA was noted in the least damaged areas. These histologic results parallel the observation that viral replication continued in thy/liv implants to day 14 and had decreased significantly by day 21 postinfection. Controls using nonimmune serum for immunohistochemical stains and using the vector alone as a probe for in situ hybridization were negative when tested with infected implant tissue (data not shown).

FACS analysis of T cells from thy/liv implants. To identify the target cell populations that supported VZV replication in the thy/liv implants, cells from four implants infected with wild-type or Oka strain and from uninfected controls were obtained at day 7 postinoculation and analyzed by FACS using polyclonal VZV-immune serum. FACS analysis showed that viral protein expression was present in all T-cell subpopulations, including CD4⁺, CD8⁺, and CD4⁺ CD8⁺ cells (Fig. 3). The percentages of infected T cells within each subpopulation were comparable for wild-type and Oka VZV strains. VZV protein expression reached a peak of 10 to 30% in each T-cell subpopulation by day 7 postinfection and declined thereafter. Uninfected control cells incubated with VZV-immune immunoglobulin G antibodies had fluorescence similar to the average background levels of 2.4 to 4.5% seen when infected cells were incubated with nonimmune polyclonal human immunoglobulin G.

The total number of lymphocytes in infected thy/liv implants decreased more than 10-fold by 7 days after inoculation, although the relative proportion of each T-cell subpopulation remained unchanged compared with control tissues (Table 1). Representative FACS plots from thy/liv implants infected with the wild-type or Oka VZV strain show that the ratios of each T-cell subpopulation varied somewhat between implants yet were not significantly different from those in uninfected implants (Fig. 4). The percentage of CD4⁺ CD8⁺ T cells expressing VZV proteins from a representative experiment that showed VZV protein expression was 7.6% for wild-type-infected cells and 28% for Oka-infected cells, with a background level of 1.7% for uninfected cells (Fig. 4). While there was some variability between implants, the differences were not significant. The stability of the T-cell distribution in conjunction with the FACS data indicated that all T-cell subpopulations were infected to similar extents and that no particular subtype was the preferred target of VZV replication.

The frequency of culturable VZV-infected cells among the sorted T-cell subpopulations was 1 to 3 cells per 10,000 (Table 1). With such an infection rate, an implant containing 4.4×10^8 lymphoid cells (the average number calculated at day 7 postinoculation) would contain from 4.4×10^4 to 1.3×10^5 PFU of infectious virus. This titer corresponds to the average of 5.0×10^4 infectious foci per implant when unsorted VZV-infected cells were cultured on day 7 postinfection (Fig. 1). The number of infectious cells in the sorted and unsorted T cells is less than the 10 to 30% of T cells expressing VZV antigens observed by FACS (Fig. 3) because the plaquing efficiency of cells in the advanced stages of infection is low.

VZV release from infected thy/liv cells. The distribution of VZV in infected thy/liv implants observed by immunohistochemistry and in situ hybridization indicated that virus spread by conventional cell fusion and also probably by transport of single particles to areas distant from the site of injection. Evidence for the release of free virus particles was obtained in the transwell assay, using VZV-infected thy/liv cells obtained at 7

FIG. 2. Histological analysis of thy/liv implants infected with VZV. Infected implants were fixed in formalin, paraffin embedded, and cut into 3- μ m sections before staining. Immunohistochemical staining on day 2 (A; magnification, $\times 1,062$) showed VZV antigen (red color) associated with lymphocytes that had been engulfed by macrophages (arrowheads). H&E staining (B; magnification, $\times 536$) revealed lymphocyte depletion, fibrosis, and infiltration of erythrocytes by day 7 postinfection. Mock-infected implants stained with H&E had a completely normal appearance (B; magnification, $\times 536$). By day 14 postinfection, in situ hybridization detected VZV DNA (dark brown color) predominantly in lymphocytes (right side of photo) and to a limited extent in the stroma (C; magnification, $\times 354$). At this time, VZV antigen was associated with most of the cells in the lymphoid lobes (D; magnification, $\times 354$), while the epithelial stromal cells were not a major site of VZV antigen production (left side of photo).

days postinfection; implant cells were separated from a Vero cell monolayer by a 3.0- μ m-pore-size membrane. VZV plaques were identified in three of three samples of 10^5 lymphocytes from infected thy/liv implants. Uninfected lymphocytes and tissue culture media alone yielded no plaques when tested in the transwell assay.

Histological analysis of VZV-infected skin implants. By day 7 postinfection, areas of the epidermis in VZV-infected skin implants showed thickening, the presence of multinucleated cells, and mononuclear cell infiltrate (Fig. 5A). The uninfected implants retained their normal structure and appearance throughout the experiment, and no in situ hybridization signal was detected (Fig. 5E and F). The severity and number of lesions were equivalent in the skin implants whether the virus was inoculated by injection or by scarification. More advanced

lesions were associated with disruption of the keratinized outer layer of the skin and with spread of the virus deeper into the dermis (Fig. 5C). In situ hybridization of these lesions revealed VZV DNA within cells where tissue damage was the most obvious, as well as in the glandular cells and fibroblasts of the dermis (Fig. 5B and D). These cutaneous lesions showed a marked increase in size by day 14 postinfection; the infection penetrated into deeper layers of the dermis, balloon cells were prevalent, and acellular material from degenerated cells was enclosed by a keratinized surface layer (Fig. 5C).

DISCUSSION

These experiments demonstrate that the SCID-hu mouse model provides an important new system for investigating

FIG. 3. FACS analysis of VZV-infected T-cells. FACS analysis was performed on lymphocytes obtained from infected implants at day 7 postinfection. The cells were treated with antibodies and fluorescent conjugates to CD4, CD8, and VZV proteins. The percentages of VZV-positive T cells of each subpopulation are shown for implants infected with either the wild-type or the Oka VZV strain. The negative controls are cells from uninfected implants treated with VZV-immune serum and the background fluorescence of infected cells treated with nonimmune human serum.

VZV interactions with cell types that are critical for VZV pathogenesis in the human host. Primary VZV infection is characterized by the occurrence of cell-associated viremia which is followed by the appearance of widely distributed, cutaneous vesicles. In the immunocompromised host, failure to terminate viremia results in fatal varicella, and viremia occurring in the course of VZV reactivation is associated with life-threatening complications of herpes zoster (3). Cell-associated viremia can be documented by the recovery of infectious VZV and by *in situ* hybridization, Southern blotting, or PCR to detect viral gene sequences. VZV tropism for human lymphocytes has been suspected on the basis of isolation of infectious virus from the nonadherent fraction of peripheral blood mono-

FIG. 4. FACS analysis of CD4, CD8, and VZV proteins on infected T-cells. FACS analysis was performed on lymphocytes obtained from infected thy/liv implants at day 7 postinfection. The cells were treated with antibodies and fluorescent conjugates to CD4, CD8, and VZV proteins. On the left, scatter plots show the CD4⁺ and CD8⁺ cells, with the percentages of each subpopulation given. On the right, the corresponding scatter plots show the forward scatter of the CD4⁺ CD8⁺ gated cell population expressing VZV proteins, which appears to the right of the line demarcating the background fluorescence. The percentages of VZV⁺ cells are given. (A) Uninfected cells; (B) wild-type-infected cells; (C) Oka-infected cells.

nuclear cells (PBMC) as well as by the morphology of PBMC that harbored viral DNA, using *in situ* hybridization, but a more precise identification of the subpopulation of PBMC involved in VZV viremia has not been possible for technical reasons (10, 13, 25, 31). In healthy individuals with varicella, VZV infects only about 1 in 100,000 PBMC (13). PCR is very sensitive for demonstrating viremia, but it cannot be used to define the cell type that is infected (14, 26).

Our experiments in which thy/liv implants in SCID-hu mice were infected with VZV show that the virus replicates very efficiently in both CD4⁺ and CD8⁺ T cells as well as immature CD4⁺ CD8⁺ T cells *in vivo*. Experiments in the SCID-hu mouse system may also explain the recovery of VZV from the adherent monocyte/macrophage fraction of PBMC from healthy subjects with varicella, since macrophages that had ingested one or more VZV-infected lymphocytes were common throughout the thymic tissue sections and could contain infectious VZV, although further experiments would be required to verify this finding (2, 19). VZV replication occurred in T cells within the environment of the thymus over a shorter time interval than would be expected following infection of permissive human fibroblast cell lines *in vitro*. The frequency of productively infected cells is very low when primary cultures of PBMC or activated T lymphocytes are inoculated with VZV *in vitro*; infection of Epstein-Barr virus-transformed B cells or Raji cell lines can be accomplished but probably has limited

TABLE 1. Proportion of T-cell subpopulations in thy/liv implants and the frequency of VZV-infected cells in each subpopulation^a

T cells	Distribution of T cells (% ± SD) ^b		PFU/10,000 cells	
	Infected	Uninfected	Infected	Uninfected
CD4 ⁺	10.4 ± 2.4	10.3 ± 1.2	2/10,000	0/10,000
CD4 ⁺ CD8 ⁺	85.1 ± 2.7	84.7 ± 1.1	1/10,000	0/10,000
CD8 ⁺	3.5 ± 1.1	4.5 ± 1.7	3/10,000	0/10,000

^a FACS analysis was used to determine the proportion of each T-cell subpopulation present in implants infected with wild-type or Oka VZV strain and in uninfected controls. Infectious VZV in sorted T-cell populations was assessed by infectious focus assay.

^b The values do not add to 100% because CD4⁺ CD8⁺ double-negative lymphocytes constitute a small fraction of the cells.

FIG. 5. Histological analysis of VZV-infected skin implants. Subcutaneous skin implants infected with VZV were fixed in formalin, paraffin embedded, and cut into 3- μ m sections before staining with H&E (A, C, and E) or in situ hybridization (B, D, and F). At day 7 postinfection, VZV lesions appeared in the epidermis (A) and VZV DNA was detected (B). The lesions had progressed by day 14 (C), and VZV DNA was detected in the lesion and deeper in the dermis (D). Mock-infected skin implants had a completely normal appearance at day 14 (E), and no VZV DNA was detected (F). Magnification, $\times 61$.

relevance to VZV pathogenesis in vivo (6, 10, 13, 16). The capacity to investigate VZV interactions with T lymphocytes in the SCID-hu model will be essential for experiments to identify VZV gene functions that account for this important tropism of the virus. The model will also permit useful comparisons of VZV T-cell tropism with the pathogenicity of other human herpesviruses for this target cell population. For example, herpes simplex virus, which is the herpesvirus most closely related to VZV, rarely causes viremia in the immunocompetent host, while human herpesvirus 6, which is not an alphaherpesvirus, appears to infect CD4⁺ T cells preferentially in healthy infants with roseola (exanthem subitum) (28).

In vivo infection of human T cells with VZV in the SCID-hu system permitted the novel and important observation that these cells release infectious virus. VZV is well known for its completely cell associated replication pattern in vitro regardless of the cell line used for its propagation; infectious virus is not released into the supernatant even when CPE is extensive.

How VZV replication in T cells facilitates release of infectious virions warrants further investigation. Release by infected lymphocytes could accelerate the spread of VZV through lymphoid and reticuloendothelial tissues that are considered to be the initial site of VZV replication during primary infection (11). Transfer of VZV to epidermal cells may be enhanced by release of virus from infected T cells trafficking through skin capillaries. Direct investigations of VZV tropism for human epidermal cell subpopulations will be also be possible in the SCID-hu mouse since typical cutaneous lesions are produced by VZV inoculation of skin implants, although the experimental lesions on skin implants are smaller than those observed clinically. Both experimentally induced and naturally occurring VZV vesicles contain balloon cells with intranuclear inclusions, multinucleated giant cells, and a fibrinopurulent exudate covered by a keratinized roof (7, 30).

Experiments in the SCID-hu model demonstrate that the Oka strain of VZV, which is used to prepare the live varicella

vaccine, is not altered in its tropism for CD4⁺ and CD8⁺ T cells. The attenuation of the Oka strain was carried out by using the classic approach of growth in nonhuman cells, which was accomplished by passing the original clinical isolate in guinea pig fibroblast cells (29). The Oka strain resembles geographically related isolates by restriction enzyme analysis; no unique molecular marker that can be associated with attenuation has been identified for the vaccine strain (8, 15). The initial replication of the Oka strain was somewhat slower than that of the wild-type strain, but the infectious virus titers and CPE were comparable at later time points. The Oka/Merck varicella vaccine has been shown to be a safe and effective vaccine against primary VZV infection in healthy susceptible children and adults through large-scale clinical trials (9). However, vaccine administration to some immunocompromised children is associated with a varicella-like rash from which the Oka strain can be recovered, indicating the occurrence of viremia after immunization (5, 9). In the context of this clinical experience, the failure to observe changes in the pathogenicity of the Oka strain in the SCID-hu model suggests that vaccine virus attenuation, which is well documented among healthy children and adults, is due to host factors rather than to significant genetic mutations in the Oka strain.

VZV infection of thy/liv implants in the SCID-hu mouse model exhibits significant differences in cell tropism compared with human cytomegalovirus. In contrast to VZV, human cytomegalovirus replicates in the thymic epithelial cells only and does not cause T-lymphocyte infection or depletion (18). Measles virus, like VZV, causes a cell-associated viremia in the course of primary infection in the human host. In the SCID-hu model, wild-type measles virus replicates within the stromal cells of the thymic epithelium and causes severe T-cell depletion by inducing apoptosis (4). While the Oka strain retained tropism for CD4⁺ and CD8⁺ T lymphocytes and the only evidence for altered pathogenicity was a somewhat slower initial replication phase, the further attenuated measles vaccine strain did not induce the pathologic effects induced by the virulent measles strain. The normal pattern of T lymphopoiesis in thy/liv implants has also allowed in vivo studies of the T-cell tropism of HIV and strain comparisons (1, 12, 27). Thus, VZV is one of several human viral pathogens that can be investigated effectively in the SCID-hu mouse system. The opportunity now exists to evaluate the replication of naturally occurring variants of VZV and genetically engineered recombinant strains of VZV in differentiated human cells in vivo.

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