Tropism of Varicella-Zoster Virus for Human Tonsillar CD4+ T Lymphocytes That Express Activation, Memory, and Skin Homing Markers

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Varicella-zoster virus (VZV) is a human alphaherpesvirus with the characteristic neurotropism of this group, but VZV also infects T cells productively and downregulates major histocompatibility complex (MHC) class I expression on infected T cells, as shown in the SCID-hu mouse model. T-cell tropism is likely to be critical for the cell-associated viremia associated with primary VZV infection. In these experiments, we found that VZV infects human tonsillar CD4+ T cells in culture, with 15 to 25% being positive for VZV proteins as detected by polyclonal anti-VZV immunoglobulin G (IgG) staining and monitored by flow cytometry analysis. RNA transcripts for VZV gE, a late gene product, were detected in T-cell populations that expressed VZV surface proteins, but not in the VZV-negative cell fraction. Exposure to phorbol myristate acetate resulted in an increase in VZV-positive T cells, indicating that viral DNA was present within these cells and that VZV gene expression could be induced by T-cell activation. By immune scanning electron microscopy, VZV virions were detected in abundance on the surfaces of infected tonsillar T cells. The predominant CD4+ T-lymphocyte subpopulations that became infected were activated CD69+ T cells with the CD45RA- memory phenotype. Subsets of CD4+ T cells that expressed skin homing markers, cutaneous leukocyte antigen, and chemokine receptor 4 were also infected with VZV. By chemotaxis assay, VZV-infected T cells migrated to SDF-1, demonstrating that skin migratory function was intact despite VZV infection. The susceptibility of tonsil T cells to VZV suggests that these cells may be important targets during the initial VZV infection of upper respiratory tract sites. Viral transport to migrating T cells in the tonsils may facilitate cell-associated viremia, and preferential infection of CD4 T cells that express skin homing markers may enhance VZV transport to cutaneous sites of replication.

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and abortive infection in T-cell lines (44) suggest that the growth of VZV is inefficient in resting, quiescent T cells or is restricted to some T-cell populations. In this paper, we report that T cells isolated from human tonsils were readily infected with VZV in vitro and produced infectious virions. We demonstrated that VZV had increased tropism for T cells of the activated, memory phenotype with skin homing markers.

MATERIALS AND METHODS

Virus and cell culture. Cosmid-derived virus Oka virus (V-Oka) was made as described previously (14, 22). Recombinant V-Oka was recovered from melanoma cells and propagated in human embryonic lung (HEL) fibroblasts in tissue culture medium containing minimal essential medium supplemented with 50 IU of penicillin, 50 μg of streptomycin, and 0.5 μg of amphotericin B (Fungizone) (Flow Laboratories, Inc., McLean, Va.) plus 10% fetal bovine serum (FBS). After three passages in HEL cells, V-Oka stocks were stored in freezing medium with 10% dimethyl sulfoxide (DMSO) at −70°C; V-Oka was grown in HEL cells for transfer to T cells and used at a maximal passage of 12.

Infection of human T cells. Human tonsil tissue was obtained according to a protocol approved by the Stanford University, School of Medicine, Human Subjects. The tonsils were dissociated to single cells, resuspended in prewarmed RPMI–10% FBS, and incubated at 37°C for at least 30 min to remove adherent cells. Nonadherent cells were loaded on a sterile nylon wool column that had been washed and soaked in Hank’s balanced salt solution (HBSS)–5% FBS at 37°C and then were incubated for 30 to 40 min at 37°C, and cells were eluted in an appropriate volume of HBSS–5% FBS. The eluted cells were stored in freezing medium with 10% DMSO at −70°C. To further purify T cells, the cells recovered from nylon wool columns were loaded onto an affinity T-cell column (Pierce, Inc., Rockford, Ill.) and eluted according to the manufacturer’s protocol; the purity of T cells was >95%.

Infection of T cells with VZV was achieved as previously described (44). Briefly, 5 × 10^6 to 10 × 10^6 column-purified T cells were resuspended in 7 ml of prewarmed RPMI–10% FBS supplemented with 4 IU of interleukin-2 (IL-2), 50 mM β-mercaptoethanol, and 10 μg of gentamicin per ml (GIBCO, Inc., Rockville, Md.). T cells were added to a monolayer of V-Oka-infected HEL cells in a T-75-cm² flask when the HEL monolayer exhibited a cytopathic effect (CPE) of 1 to 2 and incubated at 37°C for 2 to 3 days with daily changes of medium. T cells were then removed from the flask and washed before further analysis. In some experiments, peripheral blood T cells were used instead of tonsil T cells. To stimulate VZV replication in infected T cells, T cells cocultured with a VZV-infected HEL monolayer were removed and incubated with the phorbol ester phorbol myristate acetate (PMA; Promega, Inc., Madison, Wis.) at 100 nM at 37°C for 24 h. The cells were also incubated with a mitogen-activated protein kinase (MAPK) inhibitor, U0126 (Promega, Inc.), at 10 μM in 0.1% DMSO at 37°C, 30 min prior to PMA treatment. Cells from the same preparation were incubated with 0.1% DMSO as negative controls for U0126 treatment.

Antibodies and flow cytometry analysis and separation. Phycocyanin (PE), fluorescein (FL), CyChrome (CyC), PerCP, and allophycocyanin (APC)-labeled antibodies for human cell surface proteins were used in four-color analysis. These monoclonal antibodies (MAbs) included anti-CD3 (clone UCHT1); anti-CD4 (clone RPA-T4); anti-ICAM-1 (anti-CD54, clone HA58); anti-transferrin receptor (anti-CD71, clone MA712); and anti-integrin 7 (clone FIB004) (BD PharMingen, Inc., San Jose, Calif.). Additional PE- or FL-labeled MAbs included anti-CD25 (clone CD25-3G10), anti-CD38 (clone HIT-2), and anti-CD62L (clone DREG-56). Antibodies to human chemokine receptor 4 (CCR4) MAb (clone 1G1) was a gift from David Andrews (Jackson ImmunoResearch Laboratories, West Grove, Pa.) for another 30 min on ice followed by addition of other fluorochrome-conjugated MAbs to human T-cell surface markers. VZV-infected T cells were detected with IgG-purified polyclonal anti-VZV human immune serum, which binds primarily to viral glycoproteins and fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG (Caltag, Inc.) followed by staining with a panel of MAbs against human surface proteins and CD3. Normal human serum with anti-VZV IgG was used as a control for background staining. The stained cells were analyzed by FACS-Calibur with CellQuest software (Becton Dickinson, Inc., San Jose, Calif.). Subpopulations of virus-infected and uninfected T cells were separated by FACS-Vantage (Becton Dickinson, Inc.).

RNA extraction and RT-PCR. T cells were infected with VZV as described above and stained with PE-conjugated anti-human CD3 for FACS. After sorting, 5 × 10^5 to 10 × 10^5 CD3+ T cells were homogenized by incubation with 1 ml of Trizol reagent (Invitrogen, Inc., Carlsbad, Calif.) for 5 min at room temperature. Choloroform (200 μl) was added to the homogenized samples, which were then centrifuged at 12,000 rpm in a Sorvall Biofuge fresco (Kendro Laboratory Products, Newtown, Conn.) for 15 min at 4°C. RNA from the aqueous phase was precipitated with isopropanol and washed twice in 70% ethanol by centrifugation at 10,000 rpm for 5 min at 4°C. The RNA pellet was briefly air dried, resuspended in diethyl pyrocarbone-water, and stored at −20°C.

VZV gE transcripts were analyzed by reverse transcription-PCR (RT-PCR) with the SUPERSCRIPT One-Step RT-PCR with PLATINUM Taq system (Invitrogen, Inc.) according to the manufacturer’s instruction. Prior to cycling initiation, DNA contamination was removed by incubating 10 ng of template RNA with 5 × 10^5 cell equivalents with 0.1 U of DNase I (amplification grade; Invitrogen, Inc.) at room temperature for 15 min. The DNase I was inactivated by adding 1 μl of 25 mM EDTA to the mixture and heating at 65°C for 10 min. The mixture in a 50-μl reaction mixture containing 200 nM sense and antisense primers plus 2 U of RT/PLATINUM Taq mix and 2× reaction mixture was amplified as follows: 1 cycle of 50°C for 30 min and 94°C for 2 min for cDNA synthesis and predenaturation; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; and, finally, 1 cycle of 94°C for 30 s, 55°C for 30 s, and 72°C for 5 min. For the RNA control, the RT/Taq mix was replaced with 2 U of Taq mixture and amplified as described above. The oligonucleotide primer sequences of gE in the VZV Spe23 cosmid were as follows: sense, 5′-GCCGCA CGATTCCACG-3′; and antisense, 5′-CCATCGCCGATCATGTA-3′.

Infectious focus assay. T cells recovered from V-Oka-infected HEL cells were stained with anti-VZV immune serum and anti-CD3 MAb and separated into infected and uninfected CD3+ T-cell populations by cell sorting. The cells were incubated, recounted on a subconfluent monolayer of melanoma cells in 12-well plates, and incubated at 37°C for 5 to 7 days until CPE was observed. The monolayers were fixed in 4% paraformaldehyde, washed in PBS, and incubated with anti-VZV immune serum at 1:500 for 1 h at room temperature. Infectious foci were detected by adding biotinylated anti-human IgG (Vector, Inc.) for 30 min at room temperature and washing and incubation with streptavidin–alkaline phosphatase reagent (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.) for 30 min at room temperature. Plasmas were visualized by adding Fast Red substrate (Sigma, Inc.) and counted.

Chemotaxis assay. Chemotaxis assays were performed with 24-well-plate tissue culture inserts (Costar, Inc., Cambridge, Mass.) with 5-μm-pore-diameter polycarbonate filters. Recombinant human chemokine SDF-1α was purchased from PeproTech, Inc. (Rocky Hill, N.J.). T cells were cocultured with V-Oka-infected HEL cells and removed from the infected monolayer 2 days after infection. The T cells were washed in PBS and resuspended in chemotaxis medium, consisting of RPMI–0.5% bovine serum albumin (BSA). T cells (5 × 10^5 cells in 100 μl) were placed inside the insert, and 600 μl of a given chemokine dilution was added to the bottom well. The plate was incubated at 37°C for 2 h, and the inserts were removed. The cells that had migrated were harvested and analyzed for their expression of T-cell surface markers by FACS. The numbers of a particular T-cell subset that were present in the original and the migrating populations were normalized. The percentage of T cells that migrated to the SDF-1α chemokine was calculated, and results were expressed as a percentage of maximal migration (8).

Electron microscopy. Infected and uninfected T lymphocytes were fixed in 0.1% glutaraldehyde in sodium phosphate buffer (PBS) at pH 7.4 for 10 min. Samples were then pelleted down at 1,500 rpm for 15 min; lymphocytes were resuspended in PBS and gently rinsed. Resuspended lymphocytes were then blocked with 5% BSA and 1% normal goat serum for 30 min and incubated with MAB 3B3 at 37°C for 1 h. Rinsed samples were then incubated with a secondary antibody (goat anti-mouse IgG coupled to 10-nm-diameter colloidal gold particles) diluted 1:50 in PBS at 37°C for 1 h, followed by extensive washes with PBS. Labeled samples were incubated with anti-CD4 (clone MEM56), anti-CD57 (clone TB01), anti-CD62L (clone DREG-56), and anti-ICAM-1 (anti-CD54, clone HA58) at 1:50 for 30 min at 4°C, rinsed with chilled PBS, followed by postfixation with 1% osmium tetroxide in PBS at 4°C for 1 h. Samples were mounted in a poly-l-lysine-
covered glass coverslip, dehydrated in a graded ethanol series, and dried by a critical point drying method. Samples were then mounted onto aluminum plates and were observed in a high-resolution scanning electron microscope (LEO5300).

RESULTS

Expression of VZV proteins on human tonsil T cells. T cells isolated directly from tonsils were infected with VZV as judged by surface expression of VZV proteins. In more than 10 experiments, when column-purified T cells from human tonsils were incubated with VZV-infected HEL monolayers, viral proteins were detected on surfaces of 15 to 25% of CD3\(^+\) T cells incubated with polyclonal VZV IgG serum and examined by flow cytometry. A representative experiment is shown in Fig. 1. Both CD4\(^+\) and CD8\(^+\) T cells from tonsils were infected by VZV. The percentages of VZV infection from four experiments were 20.5% (range, 11 to 28%) for CD4\(^+\) T cells and 13% (range, 8 to 22%) for CD8\(^+\) T cells. More than two-thirds of CD3\(^+\) T cells recovered from tonsils were CD4\(^+\) T cells. For subsequent experiments, we focused on VZV-infected CD4\(^+\) T cells. In contrast to CD4\(^+\) T cells from tonsils, VZV infection was detected in 4 to 10% of CD4\(^+\) T cells from peripheral blood from four experiments (data not shown).

VZV gene transcription and production of infectious VZV by CD4\(^+\) T cells. Flow cytometry analysis of VZV infection detected late viral proteins on T-cell membranes, indicating that viral genes of all three classes were transcribed. However, we have observed that VZV infection of the CD4\(^+\) T-cell hybridoma cell line, designated II-23, progressed to late gene expression, but no infectious virus was produced (44). To demonstrate that VZV replicated in T cells, RT-PCR was used to detect de novo transcription of the most abundant late gene, gE, as shown in Fig. 2. RNA isolated from VZV-infected HEL fibroblasts was included as positive control for gE (Fig. 2, lanes 1 and 2). CD3\(^+\) T cells were separated by FACS after coculture with VZV-infected HEL to remove infected fibroblasts that served as an inoculum. Thirty percent of these cells were positive for gE expression by staining with anti-VZV IgG immune serum. A 1,070-bp fragment for VZV gE was amplified by RT-PCR in CD3\(^+\) T cells (50,000 cells per sample) that had been cocultured with VZV-infected HEL monolayer (Fig. 2, lanes 3 and 5), but not in uninfected CD3\(^+\) T cells (Fig. 2, lane 7). Although a small amount of viral DNA was present in the CD3\(^+\) RNA sample, it was eliminated by DNase I treatment (Fig. 2, lanes 4 and 6). The results indicated that RT-PCR amplified mRNA representing gE gene transcription. The presence of gE transcripts in the infected CD3\(^+\) T cells suggested that VZV progressed through all stages of viral replication in T cells after entry and indicated that expression of VZV proteins on the T-cell surface was due to intracellular viral synthesis rather than residual virions from the inoculum.

To further demonstrate that the T cells were productively infected with VZV as opposed to exhibiting surface attachment of virions, T cells were stimulated with PMA after coculture with a VZV-infected HEL monolayer. The expression of VZV glycoproteins by T cells was analyzed by FACS. Results from one of three such experiments are shown in Fig. 3. VZV replication in T cells was enhanced after PMA activation, since the percentage of CD3\(^+\) T cells that expressed VZV proteins increased from 17.8% in mock-treated T cells (Fig. 3A) to 43.5% in PMA-treated T cells (Fig. 3B). Furthermore, this enhancement was inhibited by U0126 (Fig. 3C), a MAPK inhibitor that blocks downstream proteins mediated by PMA activation (9). The data suggested that VZV glycoproteins expressed on T cells detected by FACS were due to transcription of VZV genomic DNA present within T cells prior to PMA activation. The results, together with RT-PCR analysis, demonstrated that VZV actively infected T cells.
To determine whether primary CD4+ T cells from tonsils were productively infected, VZV-infected T cells were tested with the infectious focus assay. VZV-infected T cells were recovered from infected HEL cell monolayers, sorted, and inoculated onto melanoma cells. One plaque per 100, 800, and 5,000 T cells was observed in three experiments in which melanoma cells were fixed and stained 5 to 7 days after inoculation with sorted T cells that expressed VZV protein. No plaques were observed in monolayers inoculated in parallel with the VZV-negative T cells from each cell preparation. The results demonstrated that CD4+ T cells were permissive for VZV replication and that infectious VZV was transferred from T cells to other permissive cells.

Detection of VZV virions on CD3+ T-cell membranes by immunogold imaging. Column-purified T cells, >95% of which were CD3+, were cocultured with a VZV-infected HEL monolayer and isolated by Ficoll centrifugation. T cells from the same preparation that did not express VZV proteins were used as a control. By scanning electron microscopy, the lymphocytes were grossly spherical, with occasional protruding microvilli. No viral particles were expressed on the surfaces of uninfected T cells (Fig. 4A). In contrast, VZV-infected T cells exhibited ruffles and microvilli on their surfaces (Fig. 4B). When viewed at higher magnification, viral particles from 150 to 200 nm in diameter also were visible on the surface of these cells (Fig. 4C). The virions were spherical, and most were found between the microvilli (Fig. 4C); occasionally, virions were also seen in small clusters (Fig. 4B). Virions exhibited specific immunogold labeling of the VZV glycoprotein gE on their envelopes (Fig. 4C). In previous studies with scanning electron microscopy (13), VZ virions were observed to emerge onto the surface of infected human melanoma cells in a distinctive pattern termed “viral highways.” The particles in the highways were pleomorphic in size and shape. In lymphocytes, the particles emerged randomly over the surface and did not form viral highways; however, the particles remained pleomorphic in size.

Relationship between CD4+ T-cell memory/naive and activation phenotypes and VZV infection. Cell surface phenotypes of CD4+ T cells from tonsils and peripheral blood were examined as background for the analysis of relative susceptibility of CD4+ T-cell subpopulations to VZV infection (Fig. 5). As expected, most CD4+ T cells from peripheral blood were CD69+ (Fig. 5A). In contrast, many tonsillar CD4+ T cells were CD69−, including naive (CD45RA+) as well as memory (CD45RA−) subpopulations. The average frequency of CD4+ T cells that expressed CD69 was 20% (range, 14 to 38%) for naive cells and 85% (range, 77 to 92%) for memory cells, respectively, in 10 tonsil specimens (Fig. 5B). Constitutive expression of CD69 on T cells in tonsils may be due to chronic exposure to cytokines and antigens in the tissues (19). Expression of transferrin receptor CD71, which is a surface marker for cellular proliferation, was also elevated on tonsillar CD4+ T cells (Fig. 5D) compared to CD4+ T cells from peripheral blood (Fig. 5C).

When the relative susceptibility to VZV infection of unfractionated CD4+ T cells that were CD45RA− or CD45RA+ was analyzed, memory T cells were consistently more likely to be VZV infected than naive T cells. A representative experiment is shown in Fig. 6. In order to further analyze this phenomenon, experiments were done in which CD4+ CD45RA− and CD4+ CD45RA+ T-cell markers were used to separate the populations into naive and memory subsets before VZV infection. When the frequency of VZV-infected T cells was assessed 2 to 3 days after inoculation, memory T cells were significantly more likely to be VZV infected than naive T cells. Although the percentages of T cells that were infected varied in five independent experiments, an increase of at least threefold was observed in memory T cells (paired t test, P < 0.05) (Table 1). Although it was not likely that VZV expression would alter CD45 isoforms, control experiments were done to show that T cells remained CD45RA+ despite being incubated with infected HEL cells (data not shown).

As shown in Fig. 5, most of the memory CD4+ T cells in tonsils were CD69−, and about 10 to 15% of these T cells also expressed CD71. To further examine the phenotypes of CD4+ T cells that were most likely to be infected with VZV, we separated naive and memory T cells into activated and nonactivated subsets, based on the expression of the CD69 activation marker. At 2 days after infection, the percentages of CD69− naive, CD69+ memory, and CD69+ memory T cells that were infected by VZV were 6, 14, and 24%, respectively. Although
the frequency of VZV-infected T cells was increased in CD69⁺ naive CD4⁺ T cells compared to cells that were CD69⁻, the percentage was consistently lower than was observed for CD69⁺ memory CD4⁺ T cells.

In order to investigate whether VZV was more likely to be detected in CD4⁺ T cells that were proliferating, memory T cells were separated into subsets based on CD71 expression. In this experiment, the percentages of VZV infection were 31.3% for CD71⁺ CD4⁺ memory T cells and 27.3% for CD4⁺ memory T cells without CD71 expression (data not shown). Taken together, these experiments indicate a hierarchy in the susceptibility of CD4⁺ T cells to VZV infection in which activated memory T cells that express CD69 are the predominant phenotype, and naive, resting T cells are least likely to have VZV proteins detected on cell membranes.

VZV infection of CD4⁺ T cells with skin homing markers and effects on chemotactic function. According to Campbell et al. (8), CCR4 is differentially expressed on CLA⁺ skin-associated (95%) and CLA⁻ intestinal-associated (20%) memory CD4⁺ T cells (8). Since the final phase of VZV replication during varicella occurs in skin, we investigated the expression of the skin homing markers CLA and CCR4 on infected CD4⁺ T cells. Results from one representative experiment are shown in Fig. 7. VZV-positive CD4⁺ T cells were consistently more likely to have CLA and CCR4 markers than VZV-negative CD4⁺ T cells. This observation might indicate that CLA⁺ or CCR4⁺ T cells are more likely to be infected with VZV or that VZV infection induces the upregulation of CLA and CCR4 on CD4⁺ T cells. Therefore, CD4⁺ T cells were separated into subsets based on CCR4 expression and incubated with VZV-
infected HEL monolayers. In these experiments, 21% of CCR4+CD4+ T cells were infected with VZV compared to 9% of CCR4−CD4+ T cells. Infection with VZV did not induce the expression of CCR4 in naive CD4+ T cells. These results indicate that T cells with skin homing phenotypes are infected preferentially by VZV.

Since expression of skin homing phenotypes might persist while chemotactic function was blocked, we tested the migration of VZV-infected CD4+ T cells in response to SDF-1α by using a standard transwell chemotaxis assay. T cells from the initial population and the cells that had migrated in response to SDF-1α were analyzed for the expression of VZV proteins and cell surface markers. Results from three independent experiments are shown in Fig. 8. In the absence of the chemokine, the mean percentages of background maximal migration for VZV infected and uninfected T cells were 2.8 and 5.2%, respectively; the percentage of infected cells in the migrated population was similar to that in the starting population. As expected, SDF-1α induced the migration of naive and memory CD4+ T cells (Fig. 8). Furthermore, chemotaxis of VZV-infected CD4+ T cells was equivalent to migration of uninfected CD4+ T cells exposed to SDF-1α (Fig. 8). These results indicate that chemotactic function is preserved in VZV-infected T cells and suggest that their skin homing potential is not impaired.

FIG. 6. Expression of VZV glycoproteins on CD45RA+ or CD45RA−CD4+ T cells. VZV infection of T cells was achieved, and the cells were then stained as described in the legend to Fig. 1. The cells were gated on CD4+ T cells and analyzed by FACS dot plots. The relationship between CD45RA expression and VZV infection in CD4+ T cells shows that more memory than naive CD4+ T cells are infected with VZV. The numbers indicated in the upper right corner are the percentages of cells in each of the corresponding quadrants.

**TABLE 1. VZV infection of naive and memory CD4+ T cells**

<table>
<thead>
<tr>
<th>Expt</th>
<th>% of cells infected with VZV in CD4+ subset</th>
<th>Memory/naive ratio</th>
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<td></td>
<td>Naive</td>
<td>Memory</td>
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<td>Mean ± SE</td>
<td>6.9 ± 1.8</td>
<td>21.0 ± 5.2</td>
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*Column-purified tonsillar T cells were stained with anti-CD4 and anti-CD45RA MAb and separated into CD4+CD45RA+ naive and CD4+CD45RA− memory T cells by cell sorting. The subpopulations were added to a monolayer of V-Oka-infected HEL cells and harvested at 2 days postinfection. The percentages of cells that had been infected with VZV were determined as described in the legend to Fig. 1. In five independent experiments, the mean percentage of memory CD4+ T cells that were VZV infected was threefold higher than that of naive CD4+ T cells.*

**Discussion**

Human herpesviruses (HHVs) infect cells of the immune system and modulate immune responses to disseminate, evade host clearance, and establish latency. For example, human cytomegalovirus infects and maintains its latency state in monocytes (41, 42). Epstein-Barr virus infects resting B lymphocytes and drives the infected cells into a differentiation pathway or leads to their elimination via programmed cell death (15, 43). Herpes simplex virus type 1 (HSV-1) infects activated cytotoxic T lymphocytes (CTLs) and destroys them through antigen-dependent fratricide (33), which may enhance virus spread from epithelium, the main cell type producing HSV-1. HHV6 and -7 predominantly infect CD4+ T cells and depend on T cells for virion production (6, 10, 20).

VZV tropism for human lymphocytes was suspected based on isolation of infectious virus from the nonadherent fraction of peripheral blood mononuclear cells (PBMCs), as well as the morphology of PBMCs that harbored viral DNA determined by in situ hybridization, but a more precise identification of the subpopulation of PBMCs involved in VZV viremia has been hampered for technical reasons (2, 11, 18, 27). Our previous experiments in which thymus/liver implants in SCID-hu mice were infected with VZV showed that the virus replicates very efficiently in both CD4+ and CD8+ T cells, as well as immature CD4−CD8+ T cells in vivo (24). These observations have demonstrated that VZV is a lymphotropic virus. Soong et al. (36) also showed that human umbilical cord blood mononuclear cells can be infected with VZV in culture and that the majority of the infected cells are T cells.

HSV, the herpesvirus most closely related to VZV, rarely causes viremia in the immunocompetent host. However, primary VZV infection is characterized by the occurrence of cell-associated viremia, which is followed by the appearance of...
widely distributed cutaneous vesicles. Hence, transport by infected lymphocytes could accelerate the spread of VZV through lymphoid and reticuloendothelial tissues, which are considered to be the initial sites of VZV replication during primary infection, to the skin. Transfer of VZV to epidermal cells may be enhanced by release of virus from infected T cells trafficking through skin capillaries. In our experiments, detection of VZV glycoproteins expressed on the surface of tonsillar T cells, as well as the presence of late gene transcripts within these cells, indicated that the virus had entered the T cells and that viral gene transcription had progressed through all three stages required for replication following entry. When these infected T cells were separated from infected HEL monolayers and cocultured with melanoma cells, viral plaques appeared on days 5 to 7 after inoculation, showing that virions made in T cells had acquired all of the viral elements needed for cell-to-cell spread. Dendritic cells can also be infected productively with VZV in vitro (1), and surface expression of VZV proteins can be detected on Epstein-Barr virus-transformed B cells and macrophages infected in vitro (reviewed in reference 2). Evaluation of the effects of mitogen stimulation on these cell types is of interest based on our observation with VZV-infected T cells.

Memory T cells acquire tissue-selective migratory capabilities after antigenic stimulation (21). They are preferentially recruited to extralymphoid sites of inflammation (7, 21). Memory T cells that express low levels of α4β7 and are CLA− tend to migrate to the skin (7, 8). CLA+ memory T cells constitute 10 to 25% of circulating CD3+ peripheral blood lymphocytes, 2 to 10% of T cells in tonsils and peripheral lymph nodes, and ~80 to 90% of T cells in most cutaneous sites of chronic inflammation (31). CLA is a carbohydrate ligand for endothelial cell-leukocyte adhesion molecule 1 (ELAM-1), which is selectively expressed on venules of inflamed skin, and functions as skin lymphocyte homing receptor (5). Binding of CLA to ELAM-1 triggers the subsequent attachment and extravasation of circulating lymphocytes from blood into peripheral lymphoid tissues. Most CLA+ memory CD4+ T cells express high levels of CCR4, which responds to its ligand, thymus-
may have an effect on the susceptibility of T cells to VZV changes in expression of surface molecules on activated T cells. Interests from a clinical perspective, because adults have much more serious varicella than young adults. Second, activated CD4+ T cells of the activated memory phenotype were more likely to be infected with VZV than resting naive CD4+ T cells. The increased susceptibility of memory CD4+ T cells is of interest from a clinical perspective, because adults have much more serious varicella than young adults. Second, activated CD69-bearing T cells had a higher susceptibility to VZV infection than the cells that did not express CD69. The differential infectivity in T cells of various phenotypes suggested that changes in expression of surface molecules on activated T cells may have an effect on the susceptibility of T cells to VZV infection. Changes in the components of the cell surface membrane could render activated memory CD4+ T cells more susceptible to VZV infection by enhancing expression of an as yet undefined receptor molecule.

T-cell activation is required for productive infection with several viruses. Infection of resting T cells with HHV6 gives no infectious virions, but lytic infection can be induced in infected T cells after phytohemagglutinin activation in tissue culture (10). Although HIV-1 enters resting naive CD4+ T cells, subsequent virus replication is blocked at nuclear import of viral DNA and results in a nonproductive infection. In contrast, productive replication is induced after T cells are activated with mitogens or anti-CD3 or anti-CD28 MAbs, possibly via activation of MAPKs (37, 39, 40). VZV IE62 protein, which is the predominant tegument protein and the major VZV-transactivating factor, is required for rapid induction of viral protein synthesis (16, 26, 30). The product of open reading frame 47 (ORF47) is a serine-threonine protein kinase (25, 38) that specifically phosphorylates IE62 to modify its function and nuclear translocation (26). A mutant VZV strain deficient in the ORF47 putative kinase was unable to replicate in the SCID-hu mouse model (24) or in peripheral blood T cells in vitro (36). The block in the infectivity of ORF47-deficient VZV for T cells may be due to impaired IE62 nuclear translocation or the absence of ORF47-mediated phosphorylation of other viral or cellular proteins to induce conditions favorable for viral replication. Our experiments showed that PMA induced virally infected T cells to express VZV glycoproteins, suggesting that T-cell activation might stimulate viral replication, either by enhancing phosphorylation and nuclear translocation of IE62 or by transactivating transcription of multiple viral genes. Experiments with PMA suggest that VZV replication in T cells may depend on functions of viral gene products after entry, rather than upon upregulation of an entry molecule on the T-cell surface.

T-cell infection is critical for the viremic phase of VZV pathogenesis. Although lymphopenia is observed in patients during the last few days of the incubation period, the total leukocyte counts rise rapidly, beginning on the 2nd and 3rd days of rash, and viremia is resolved (2). The findings in this analysis of VZV T-cell tropism have led us to hypothesize that VZV may target T cells in tonsils for infection shortly after inoculation of respiratory mucosa. The infected T cells could transfer to regional lymph nodes that contain many T cells of activated phenotypes and then emerge into the circulation, where they need to survive only long enough to reach skin sites of replication. VZV infection of activated, memory T cells, which include the skin-migratory population, may enhance the interaction of infected T cells with capillary endothelium and facilitate transfer of the virus to dermis and epidermis, initiating the cutaneous phase of primary VZV infection.

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