We sought to investigate the effects of varicella-zoster virus (VZV) infection on gamma interferon (IFN-\(\gamma\))-stimulated expression of cell surface major histocompatibility complex (MHC) class II molecules on human fibroblasts. IFN-\(\gamma\) treatment induced cell surface MHC class II expression on 60 to 86% of uninfected cells, compared to 20 to 30% of cells which had been infected with VZV prior to the addition of IFN-\(\gamma\). In contrast, cells that were treated with IFN-\(\gamma\) before VZV infection had profiles of MHC class II expression similar to those of uninfected cell populations. Neither IFN-\(\gamma\) treatment nor VZV infection affected the expression of transferrin receptor (CD71). In situ and Northern blot hybridization of MHC II (MHC class II DR-\(\alpha\)) RNA expression in response to IFN-\(\gamma\) stimulation revealed that MHC class II DR-\(\alpha\) mRNA accumulated in uninfected cells but not in cells infected with VZV. When skin biopsies of varicella lesions were analyzed by in situ hybridization, MHC class II transcripts were detected in areas around lesions but not in cells that were infected with VZV. VZV infection inhibited the expression of Stat 1 and Jak2 proteins but had little effect on Jak1. Analysis of regulatory events in the IFN-\(\gamma\) signaling pathway showed that VZV infection inhibited transcription of interferon regulatory factor 1 and the MHC class II transactivator. This is the first report that VZV encodes an immunomodulatory function which directly interferes with the IFN-\(\gamma\) signal transduction via the Jak/Stat pathway and enables the virus to inhibit IFN-\(\gamma\) induction of cell surface MHC class II expression. This inhibition of MHC class II expression on VZV-infected cells in vivo may transiently protect cells from CD4\(^+\) T-cell immune surveillance, facilitating local virus replication and transmission during the first few days of cutaneous lesion formation.

Major histocompatibility complex (MHC) class II molecules are highly polymorphic heterodimers consisting of an \(\alpha\) and \(\beta\) chain which present exogenous peptides to CD4\(^+\) T lymphocytes. The \(\alpha\) and \(\beta\) chains form a heterodimer in the endoplasmic reticulum, and this complex, when associated with the invariant chain (II), is transported through the Golgi and trans-Golgi reticulum to cytosolic endosomes. At this site, limited II chain proteolysis occurs and results in a complex of \(\alpha\)β with Ii-derived peptides, termed CLIPs (class II invariant-chain peptides). At an endosomal site, CLIP is exchanged for antigenic peptides derived by proteolysis of endocyted proteins, a process which is improved by HLA-DM molecules. The peptide-loaded MHC class II molecule is then presented on the cell surface (10, 39, 42). Constitutive expression of cell surface MHC class II is restricted to specialized cell types including B cells, monocytes, dendritic cells, and those of thymic epithelium, yet gamma interferon (IFN-\(\gamma\)) has been shown to be a potent inducer of MHC class II expression on many cell types, including fibroblasts (14, 44).

Varicella-zoster virus (VZV) is a human herpesvirus that causes varicella (chickenpox) as the primary infection in susceptible individuals, establishes latency in sensory ganglia, and may reactivate as herpes zoster (shingles) (4, 13). Multiple components of the innate and antigen-specific immune responses are activated during the course of primary VZV infection. The early host responses to VZV are nonspecific and involve natural killer cells and interferons which function to restrict virus replication and spread (2, 3, 51). VZV-specific T-cell recognition is critical for host recovery from varicella, and both MHC class I-restricted CD8\(^+\) and MHC class II-restricted CD4\(^+\) T cells are sensitized during primary VZV infection. VZV-specific CD4\(^+\) T cells that are elicited during primary infection are predominantly of the Th1 type (7, 54) and function to produce high levels of IFN-\(\gamma\), which potentiates the clonal expansion of VZV-specific T cells (3, 26, 51). Although the classical cytotoxic T-lymphocyte (CTL) response is mediated by CD8\(^+\) T cells that recognize viral peptides in association with MHC class I molecules, VZV-specific CTLs can also exhibit MHC class II (CD4\(^+\))-restricted killing of infected target cells (15, 17, 19, 20, 23, 25, 49). Based on these observations, immunomodulatory mechanisms that limit the initial presentation of VZV peptides by MHC class I or class II pathways are likely to have an important effect on viral pathogenesis.

It has been postulated that interference with MHC class II-restricted T-cell recognition may promote viral infection in the host by enabling virus-infected cells to resist a crucial arm of the immune response (38, 45). In this respect, several viruses including adenovirus, murine and human cytomegalovirus (MCMV and HCMV), mouse hepatitis virus, human immunodeficiency virus, Kirsten murine sarcoma virus, and measles virus have been shown to inhibit IFN-\(\gamma\)-mediated transcription (21, 29, 37), although posttranscriptional modulation has also been described (18, 28).

In this study, we found that VZV inhibits IFN-\(\gamma\)-mediated
induction of cell surface MHC class II expression on infected human fibroblasts. We applied a combination of reverse transcriptase (RT)-mediated PCR (RT-PCR), Northern and Western blot analyses, and in situ hybridization to demonstrate that VZV interferes with the IFN-γ signal transduction pathway to block MHC class II transcription. Examination of skin biopsies of early varicella and herpes zoster lesions from healthy adults by in situ hybridization showed that MHC class II transcripts were not detected in infected cells.

MATERIALS AND METHODS

Cells and viral culture. Human foreskin fibroblasts (HFF) were grown in tissue culture medium (TCM; Dulbecco’s modified Eagle’s medium; Gibco, Gaithersburg, Md.) supplemented with heat-inactivated fetal calf serum, 2 mM l-glutamine (Gibco), 50 IU of penicillin, 50 μg of streptomycin (ICN Biomedicals, Inc., Calif.), and 0.5 μg of amphotericin B (Fungizone; Flow Laboratories, McLean, Va.). 8.16 cells are clonally derived B-lymphoid cells grown in RPMI 1640 (Gibco)-containing TCM (32). A fresh clinical isolate, designated strain Schenke by adding VZV-infected cells to an uninfected cell monolayer at a ratio of 800:1, was used for all experiments. HFF were infected with VZV strain Schenke by mixing VZV-infected cells to an uninfected cell monolayer at a ratio of 1 infected cell to 5 uninfected cells for 2 h at 37°C. The infected monolayers were washed three times with phosphate-buffered saline (PBS), incubated with TCM for 12 h, and then incubated with TCM containing human IFN-γ (100 U/ml) for a further 36 h.

Flow cytometry. Cells were harvested, and aliquots of approximately 10⁶ cells obtained from mock- or VZV-infected cells were washed and resuspended in 100 μl of FACS (fluorescence-activated cell sorting) staining buffer (PBS with 1% FCS and 0.02% sodium azide). Primary antibodies, VZV-immune or nonimmune polyclonal immunoglobulin G, were diluted 1:40; secondary antibodies, anti-MHC class II DR- or anti-CD71, were diluted 1:20; goat anti-human FITC was diluted 1:100. As a negative control, cells were incubated with appropriate isotype controls. All antisera were inactivated by heating in FACS staining buffer (PBS with 0.5% sodium azide) for 30 min, and all reactions were done in the dark on ice for 30 min. The cells were washed between each antibody step with 2 ml of FACS staining buffer. After the final wash, cells were resuspended either in orthophosphate (PBS with 1% EM-grade formaldehyde) or in PBS for FACS sorting. Compensations were generated using a Becton Dickinson FACSAPPleuror or sorted by FACS (Becton Dickinson, San Jose, Calif.).

In situ hybridization for VZV IE62 and MHC class II transcripts. Cell populations were fixed with FACS staining buffer and resuspended in PBS before being fixed in 4% paraformaldehyde for 30 min at room temperature, washed twice in PBS for 5 min, and treated with 1% Triton X-100 in PBS for 2 min. Tissue sections (10 μm) were dehydrated in graded ethanol solution (100, 70, and 50%), and washed in PBS. To improve access of probe to target sequences, tissue sections were incubated with protease K (10 μg/ml) for 10 min at 37°C in 200 mM Tris (pH 7.5)–2 mM CaCl₂. Cell cytopsins and tissue sections were then washed twice in PBS, treated with 0.25% acetic anhydride–0.1 M triethanolamine for 10 min, washed in PBS, and dehydrated through grades (50, 70, 90, and 100%) ethanol. 20 μl of hybridization buffer (50% denaturated formamide, 1× SSC [0.15 M NaCl plus 0.015 M sodium citrate], 100 mM Tris-HCl [pH 7.6], 10 mM NaH₂PO₄, 10 mM Na₂HPO₄, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 500 μg of sheared denatured salmon sperm DNA per ml, 50 μM each triphosphatase transcriptase, 1 μl of streptomycin, 10 μl of a strand-specific deoxigenin [DIG]-labeled riboprobe generated from pBS-62C or pBS-DRαC) was added to each section, covered with a saponified coverslip, sealed with rubber cement, and hybridized for 16 h at 35°C. Sections were then washed for 5 min each in 2× SSC–10 mM Tris-HCl (pH 7.5) and 0.1× SSC–10 mM Tris-HCl (pH 7.5) for 30 min at room temperature followed by a stringent wash for 30 min at 55°C in 0.1× SSC–10 mM Tris-HCl (pH 7.5–30%) denaturated formamide. Finally, sections were washed for 15 min at room temperature in 0.1× SSC–10 mM Tris-HCl (pH 7.5), and bound probe was detected using anti-DIG antibody coupled to alkaline phosphatase and developed with nitroblue tetrazolium chloride plus 5-bromo-4-chloro-3-indolyl phosphate according protocol of the manufacturer (Boehringer, Mannheim, Germany).

Northern blot hybridization. Total RNA was isolated using a commercial kit (Ambion, Austin, Tex.), and 2.5-μg samples were separated on a 2% agarose–2% formaldehyde gel before being transferred to a nylon membrane. Membranes were incubated for 4 h at 42°C in prehybridization solution (5× Denhardts solution, 5× SSC, 0.1% sodium dodecyl sulfate [SDS], 50% denaturated formamide, 20 μg of sheared and denatured salmon sperm DNA per ml, 50 mM NaH₂PO₄, 50 mM Na₂HPO₄) before being hybridized for 16 h at 42°C in a hybridization solution (1× Denhardts solution, 5× SSC, 0.1% SDS, 50% denaturated formamide, 100 μg of sheared and denatured salmon sperm DNA per ml, 10% dextran sulfate) and random-primer 32P-labeled probe. Unbound material was removed by washing at 60°C (twice for 45 min each time) and 0.1× SSC–0.1% SDS (twice for 45 min each time), and bound probe was detected by autoradiography. Random-primer probes were generated either from pBS-DRα or from products derived from RT-PCR and were radiolabeled with 32P using a random prime DNA labeling kit (Boehringer) according to manufacturer’s directions.

Western blot analysis. Cells were washed once in PBS, sonicated for 1 min in cell extract buffer containing protease inhibitors (50 mM Tris [pH 7.4], 240 mM NaCl, 50 mM NaF, 10 mM EDTA, 1 mM NaVO₃, 0.5% phenylmethylsulfonyl fluoride), and centrifuged (14,000 × g, 5 min), and supernatants were collected. The cell supernatants (5 × 10⁶ cells/lane) were separated by SDS-polyacrylamide gel electrophoresis (PAGE) in 7% gels, followed by electroblotting to Immobilon-P polyvinylidene difluoride membranes (Millipore, Bedford, Mass.). Membranes were stained with amido black (1% amido black [naphtho; blue black], 45% methanol, 10% acetic acid) to reveal total protein before Western blot analysis. Membranes were incubated in blocking solution (5% nonfat milk in PBS) for 1 h. Jak1, Jak2, Stat 1, and CD71 proteins were detected with appropriate antibodies diluted at 1:100, 1:100, 1:250, and 1:500, respectively, in blocking solution. Secondary goat anti-rabbit or goat anti-mouse immunoglobulin G–horseradish peroxidase conjugates (Amersham, Buckinghamshire, England) were diluted 1:2,000 and used for enhanced chemiluminescence (ECL) detection of bound antibodies according to the protocol of the manufacturer (Amersham). Molecular weights of proteins were determined using protein reference standards (Bio-Rad, Hercules, Calif.);

Human skin biopsy sections. Skin biopsies of VZV lesions from healthy adult donors were collected within 4 to 9 h after the onset of varicella or herpes zoster rash, fixed in 10% formalin, and embedded in paraffin, and 10-μm sections were collected on poly-L-lysine-coated glass microscope slides. Specimens were collected with the informed consent of the donors.

RESULTS

VZV inhibits IFN-γ-induced MHC class II expression. We assessed HFF and FACS analysis to investigate the effect of VZV infection on IFN-γ-stimulated MHC class II expression. Fibroblasts do not constitutively express MHC class II but rather can be induced to express MHC class II by treatment with IFN-γ. HFF were infected with VZV strain Schenke by mixing VZV-infected and uninfected cells at a ratio of 1:5. Twelve hours postinfection, cells were treated with human IFN-γ (100 U/ml)
for 36 h to stimulate MHC class II expression, stained for VZV and MHC class II expression using polyclonal VZV-immune serum and mouse monoclonal antibody to MHC class II (MHC class II DR-α), respectively, and analyzed by FACS (Fig. 1A). Negative controls included mock-infected cells and incubation of both mock- and VZV-infected cells with isotype control antibodies. At 48 h postinfection, 32% of the cells tested positive for VZV (i.e., were VZV^+), and 68% of the cells remained VZV^- as determined by FACS. Of the VZV^- cell population, 86% expressed MHC II (i.e., were MHC II^+). In contrast, only 26% of VZV^+ cells were MHC II^+ (Fig. 1C). To ensure that the inhibition of MHC class II expression on VZV-infected cells following IFN-γ treatment was selective and not due to a general effect on host cell surface molecules, these cells were also assessed by FACS for transferrin receptor (CD71) expression, using an anti-CD71 monoclonal antibody (Fig. 1B). At 48 h postinfection, more than 98% of both VZV^- and VZV^+ cell populations expressed transferrin receptor.

Taken together, these data show that VZV selectively inhibits IFN-γ induction of MHC class II cell surface expression. In a total of five experiments, this specific inhibition of MHC class II was consistent with the mean ± standard error for MHC II^+ cells in the VZV^- cell population being 26.3% ± 2.5%, whereas of the VZV^- cell population, 74.8% ± 5.1% were MHC class II positive.

To determine whether VZV downregulates MHC class II expression on fibroblasts that were pretreated with IFN-γ, HFF were treated with IFN-γ (100 U/ml) for 36 h before being infected with VZV strain Schenke. After 48 h, the cells were analyzed by immunofluorescent staining and FACS as described above (Fig. 1D). At 48 h postinfection, 83% of both VZV^- and VZV^+ cell populations expressed MHC class II (Fig. 1F). In four separate experiments, the mean ± standard error of MHC class II positive cells was 85.0% ± 6.5% of the VZV^- population, compared with 82.0% ± 8.6% of the VZV^- population. These data indicate that VZV inhibits IFN-γ induction of MHC class II cell surface expression but does not downregulate MHC class II surface expression on cells treated with IFN-γ before infection.

**VZV inhibits IFN-γ induced MHC class II RNA expression.**

IFN-γ induction of MHC class II expression occurs at the level of gene transcription (8). We therefore evaluated the effect of VZV infection on IFN-γ-induced upregulation of MHC class II RNA expression, using both Northern blot and in situ hybridization. Twelve hours after infection with VZV strain Schenke, HFF were treated with IFN-γ (100 U/ml) and incubated for a further 36 h. Cells were stained with antibodies to MHC class II (MHC class II DR) and VZV proteins and sorted by FACS into VZV^- and VZV^+MHC class II DR-α^- cell populations. Of the VZV^+ cells, typically 70 to 85% were identified as MHC class II DR^- by FACS. Cells were subjected to total RNA extraction and analyzed by Northern blot hybridization or were cytospun onto microscope slides and analyzed by in situ hybridization.

Samples of total RNA (2.5 μg) were separated by agarose gel electrophoresis and subjected to Northern blot hybridization using an MHC class II DR-α-specific random-primed ^32^P-labeled probe derived from pBS-DR-α. Positive controls were
8.1.6 cells and mock-infected HFF treated with IFN-\(\gamma\) (100 U/ml) for 48 h. Negative controls were mock-infected and VZV-infected HFF which had not been treated with IFN-\(\gamma\). Northern blot hybridization for MHC class II DR-\(\alpha\) (A) and CD71 (B) was performed on total cell RNA extracted from 8.1.6 cells (lane 1), uninfected HFF (lane 2), uninfected HFF treated with IFN-\(\gamma\) (lane 3), VZV-infected HFF (lane 4), and HFF exposed to VZV, treated with IFN-\(\gamma\), and sorted by FACS into VZV\(^+\) (lane 5) and VZV\(^+/\)MHC class II DR\(^-\) (lane 6) cell populations.

FIG. 2. Detection of MHC class II DR-\(\alpha\) and CD71 mRNA by Northern blot hybridization in VZV-infected cells treated with IFN-\(\gamma\). Northern blot hybridization for MHC class II DR-\(\alpha\) (A) and CD71 (B) was performed on total cell RNA extracted from 8.1.6 cells (lane 1), uninfected HFF (lane 2), uninfected HFF treated with IFN-\(\gamma\) (lane 3), VZV-infected HFF (lane 4), and HFF exposed to VZV, treated with IFN-\(\gamma\), and sorted by FACS into VZV\(^+\) (lane 5) and VZV\(^+/\)MHC class II DR\(^-\) (lane 6) cell populations.

MHC class II DR-\(\alpha\) transcripts (1.6 kb) were detected in the VZV\(^+\) cells treated with IFN-\(\gamma\) as well as 8.1.6 cells and mock-infected cells treated with IFN-\(\gamma\). In contrast, VZV\(^+/\)MHC class II DR\(^+\) cells and untreated mock or VZV-infected cells did not express detectable MHC class II DR-\(\alpha\) transcripts (Fig. 2A). To determine whether the inhibition of MHC class II DR-\(\alpha\) mRNA expression in VZV-infected cells treated with IFN-\(\gamma\) was due to a general effect on host mRNA, we evaluated expression of CD71 mRNA after stripping the nitrocellulose filter of bound probe and repробing with a random-primed \(^{32}\)P-labeled probe derived from a partial-length CD71 cDNA. The probe hybridized similarly to all RNA samples (Fig. 2B), indicating that VZV specifically inhibited IFN-\(\gamma\)-stimulated expression of MHC class II DR-\(\alpha\) transcripts.

Sorted populations of cells infected with VZV and treated with IFN-\(\gamma\) were analyzed by in situ hybridization, with either strand-specific DIG-labeled riboprobes designed to detect MHC class II (MHC class II DR-\(\alpha\)) transcripts or VZV IE62 transcripts. MHC class II DR-\(\alpha\) transcripts were readily detected in the majority of VZV\(^+\) FACS-sorted cells (2,620/3,080; 85\%) (Fig. 3A) but were not detected in VZV\(^+/\)MHC class II DR\(^+\) cells (0/1,696; 0\%) (Fig. 3B). VZV probe hybridized to the majority of VZV\(^+/\)MHC class II DR\(^-\) sorted cells (1,624/1,936; 84\%) (Fig. 3C) but only sporadically to those defined by FACS as VZV\(^+\) (92/2,230; 4\%) (Fig. 3D). No staining was detected in either cell population after hybridization to a control riboprobe generated in the orientation opposite that used to detect MHC class II (MHC class II DR-\(\alpha\)) transcripts (data not shown). MHC class II transcripts were undetectable in VZV\(^+\) cells which did not express cell surface MHC class II.

MHC class II transcription in varicella skin lesion biopsies. To confirm the relevance of observations about VZV effects on MHC class II expression in HFF, we assessed the distribution of MHC class II-positive cells in skin biopsies taken from individuals who had acute varicella or herpes zoster. To determine whether VZV-infected cells express MHC class II in vivo, we performed nonisotopic in situ hybridization for MHC class II and VZV IE62 transcripts. Skin biopsies of VZV lesions from two healthy donors were taken 4 h after onset of varicella and 96 h after onset of herpes zoster. Consecutive 10-\(\mu\)m paraffin-embedded sections were collected onto slides and hy-
bridized with strand-specific DIG-labeled riboprobes designed to detect MHC class II DR-α and VZV IE62 transcripts. VZV IE62 transcripts were readily detectable within cells where tissue damage was most obvious, as well as in the glandular cells and fibroblasts of the dermis (Fig. 4A). In contrast, on the consecutive tissue sections, MHC class II DR-α transcripts were detected only in infiltrating inflammatory cells. MHC class II DR-α transcripts were detected in cells in proximity to VZV+ cells but were never detected in VZV− cells (Fig. 4B and C). In addition, a riboprobe generated in the orientation opposite MHC class II DR-α transcripts did not hybridize to consecutive sections, confirming the RNA specificity of the MHC class II DR-α staining. These data indicate that MHC class II transcripts are not expressed in VZV-infected cells in vivo, but are expressed in cells proximal to those which are infected, during the initial phase of cutaneous lesion formation.

VZV inhibits IFN-γ-stimulated CIITA expression. CIITA is induced by IFN-γ and is essential for MHC class II gene expression (11, 50). Since we did not detect MHC class II DR RNA in VZV-infected IFN-γ-treated cells, we hypothesized that CIITA expression may be deficient in these cells. To address this hypothesis, we assessed CIITA RNA expression by RT-PCR in FACS-sorted populations of cells infected with VZV and treated with IFN-γ.

Cells were infected with VZV, treated with IFN-γ, antibody stained as previously described, and FACS sorted into two populations: cells that remained VZV−, and those that were VZV+/MHC class II+. Total RNA (200 ng) was reverse transcribed with oligo(dT), and cDNAs were subjected to 30 rounds of PCR amplification with CIITA-specific primers. Controls for reverse transcription and PCR included no RT and no DNA, respectively. Following amplification, 20% of each reaction product was separated on 2% agarose gels and stained with ethidium bromide (Fig. 5A). A 700-bp product was readily visualized in samples from VZV− cells; in contrast, no CIITA sequences were detected in VZV+/MHC class II− cell samples. To ensure that the inability to detect CIITA expression in VZV-infected cells treated with IFN-γ was not due to a general effect on host mRNA, expression of CD71 RNA was evaluated by using the same cDNA samples and subjecting them to 30 rounds of PCR amplification with CD71-specific primers. In all samples, CD71-specific sequences were amplified (Fig. 5A). These data indicate that VZV specifically inhibited IFN-γ-induced CIITA expression.

VZV inhibits IFN-γ-stimulated IRF-1 expression. IRF-1 is also required for transactivation of MHC class II in response to IFN-γ (24). To determine the effect of VZV infection on IRF-1 expression, we assessed RNA levels by Northern blot hybridization. Cells were infected, antibody stained, and sorted by FACS into VZV− and VZV+/MHC class II DR-α− cell populations as described earlier. The positive control consisted of mock-infected HFF treated with IFN-γ (100 U/ml) for 36 h;

FIG. 4. MHC class II DR-α and VZV IE62 RNA expression in cutaneous varicella skin lesions. Biopsies of skin lesions from subjects with varicella were sectioned and hybridized with strand-specific DIG labeled riboprobes to VZV IE62 transcripts (A) and MHC class II DR-α transcripts (B and C). Positive hybridization for VZV IE62 was detected in the lesion and deeper in the dermis (black arrows), whereas MHC class II DR-α was detected in areas of infiltrating cells (boxed area) adjacent to VZV+ cells.

FIG. 5. Analysis of CIITA and IRF-1 RNA expression in VZV-infected IFN-γ-treated cells. (A) RT-PCR for CIITA and CD71 was performed on total cell RNA extracted from VZV-infected HFF treated with IFN-γ, antibody stained, and sorted by FACS into VZV− (lanes 2 and 3) and VZV+/MHC class II DR-α− (lanes 4 and 5). No DNA was included as a PCR control (lane 1); no-RT control for each sample is shown in lanes 3 and 5. (B) Northern blot hybridization for IRF-1 was performed on total cell RNA extracted from 8.1.6 cells (lane 1), uninfected HFF (lane 2), uninfected HFF treated with IFN-γ (lane 3), VZV-infected HFF (lane 4), and VZV-infected HFF treated with IFN-γ, antibody stained, and sorted by FACS into VZV− (lane 5) and VZV+/MHC class II DR-α− (lane 6) cell populations.
negative controls were mock- or VZV-infected HFF which had not been treated with IFN-γ and 8.1.6 cells. Total RNA (2.5 μg) was resolved by gel electrophoresis, transferred to nitrocellulose, and hybridized to 32P-labeled probe specific for IRF-1. Figure 2B shows the result of stripping and reprobing the nitrocellulose membrane depicted in Fig. 2. IRF-1 RNA (2 kb) was detected in IFN-γ-treated mock-infected and VZV−sorted cells but not in VZV+/MHC class II DR−α−sorted cells, 8.1.6 cells, or mock- or VZV-infected HFF which were not treated with IFN-γ. These data demonstrate that IRF-1 transcription is inhibited in VZV-infected cells which do not express cell surface MHC class II.

**VZV restricts IFN-γ induction of Jak2 and Stat 1α.** CITTA and IRF-1 expression is induced after IFN-γ treatment by Stat 1α (35, 43). Stat 1α is a component of the Jak/Stat signal transduction pathway, which includes Jak1 and Jak2. The failure to detect CITTA- and IRF-1 RNA in VZV-infected IFN-γ-treated cells suggested that these cells may have a disruption of the Jak/Stat pathway. To address this hypothesis directly, we used Western blotting to assess the expression of Jak1, Jak2, and Stat 1α protein in FACS-sorted populations of cells infected with VZV and treated with IFN-γ.

Cells were infected, antibody stained, and sorted by FACS into VZV− and VZV+/MHC class II DR− populations as previously described. Cell lysates were prepared, and total protein from 5 × 10⁴ cells was analyzed by SDS-PAGE and Western blotting in three separate experiments. Membranes were reacted with antibodies to Jak1, and bound antibody was visualized with an ECL detection system (Fig. 6). In both VZV− and VZV+/MHC class II DR− cell populations, levels of Jak1 protein expression showed little or no change. Membranes were stripped of bound antibody and reacted with antibody to Jak2, stripped again of bound antibody, and then reacted with antibody to Stat 1α. In contrast to the expression levels of Jak1, both Jak2 and Stat 1α protein expression was significantly reduced in VZV+/MHC class II DR−α− cells compared to VZV− cells. Stripped membranes were also reacted with antibody to CD71, which confirmed equivalent protein loading. The reduction of Jak2 and Stat 1α protein levels in VZV+/MHC class II DR−α− cells was observed in a further two experiments. These data demonstrate that VZV infection interferes with the Jak/Stat signal transduction pathway by reducing steady-state levels of Jak2 and Stat 1α but not Jak1 protein expression in IFN-γ-treated cells.

**DISCUSSION**

These experiments demonstrate that VZV encodes an immunomodulatory function which enables the virus to inhibit the induction of MHC class II expression by IFN-γ. The persistence of VZV as a human pathogen depends on its transmission from the cutaneous lesions that are associated with varicella, caused by primary VZV infection, and herpes zoster, which results from reactivation of the virus from neuronal sites of latency (4). The ability of VZV to inhibit MHC class II expression in most infected human fibroblasts, despite exposure to high concentrations of IFN-γ, provides a mechanism by which the virus can limit the consequences of immune surveillance by CD4+ T cells. Impaired recognition of VZV-infected cells antigen by CD4+ T cells, which requires interaction of the T-cell receptor and viral peptides complexed with MHC class II molecules, can be predicted to allow transient viral replication in dermal and epidermal cells that is necessary for VZV transmission to susceptible individuals.

With regard to the mechanism of inhibition of IFN-γ-induced MHC class II gene expression, we found that VZV infection inhibits IFN-γ-dependent transcription of the MHC class II DR−α− gene. HCMV and MCMV also inhibit MHC class II expression at the level of transcription (26, 37). In our studies, MHC class II DRα, CITTA, and IRF-1 transcripts did not accumulate in VZV-infected cells after treatment with IFN-γ. Stat 1α and Jak2 protein synthesis was reduced compared with Jak1 and CD71 synthesis, which remained unchanged. These observations indicate that the pathway by which VZV infection alters induction of MHC class II by IFN-γ differs from the effects of HCMV and MCMV. HCMV inhibits MHC class II expression in human fibroblasts by blocking Jak/Stat signal transduction through a specific decrease in Jak1 expression. Interestingly, the adenovirus E1A protein inhibits MHC class II expression in HeLa cells stimulated by IFN-γ at the level of Jak/Stat signal transduction by specifically decreasing Stat 1α expression (30). In contrast, MCMV inhibits IFN-γ-stimulated MHC class II expression in murine macrophages by a mechanism that does not involve Jak/Stat signal transduction. Thus, among the herpesviruses, VZV, HCMV, and MCMV employ different strategies to reduce MHC class II antigen presentation pathways.

The CD4+ T-cell response to VZV is predominantly of the Th1 type, with IFN-γ being the major cytokine produced (26). Despite the prolonged 10- to 21-day incubation period, VZV-specific T cells are usually not detected until 24 to 72 h after the appearance of cutaneous varicella lesions. The kinetics of the appearance of VZV-specific T cells suggests that their sensitization requires the replication of VZV in skin cells (5). Individuals who develop T cells that proliferate and release IFN-γ within 72 h are likely to experience mild primary VZV infection, whereas delayed acquisition of these responses is associated with more extensive cutaneous disease and the risk of progressive varicella (3, 6). A viral immunomodulatory effect that slows the initial clonal amplification of antigen-specific CD4+ T-cell populations which is enhanced by IFN-γ is likely to facilitate VZV replication at cutaneous sites transiently. VZV-specific CD4+ T cells mediate MHC class II-restricted lysis of autologous targets that express VZV envelope and structural proteins (49). Although CD8+ CTL are also induced, VZV encodes a viral gene product that causes downregulation of MHC class I expression (1). Herpes simplex virus (HSV) is the herpesvirus most closely related to VZV.
Like VZV infection, HSV infection induces CD4\(^+\) T cells that mediate cytotoxicity against HSV-infected targets. HSV-specific CD4\(^+\) CTL have been considered a potential alternative mechanism for clearing virus-infected cells since HSV inhibits MHC class I expression and impairs the cytotoxic potential of CD8\(^+\) T cells (53). Our experiments suggest that the alphaherpesviruses have also evolved mechanisms to minimize the cytotoxic potential of CD4\(^+\) T cells by limiting the induction of MHC class II expression by IFN-\(\gamma\). When VZV reactivates, the capacity of viral gene products to block the upregulation of MHC class II expression triggered by IFN-\(\gamma\) should permit a sufficient period of viral replication to cause the lesions of herpes zoster despite the presence of circulating VZV-specific, memory CD4\(^+\) T cells in the immune host. The transmission of VZV from older individuals with herpes zoster causes varicella in the naive contact and is critical for preservation of the virus in the human population.

The significance of the in vitro studies is substantiated by examination of human skin biopsies for MHC class II and staining with inflammatory cells, suggesting that this effect may transiently protect infected cells from CD4\(^+\) T-cell immune surveillance. Similar mechanisms for VZV and HSV are likely to be involved in the immune response to VZV and HSV.

In conclusion, we have demonstrated that VZV infection inhibits MHC class II expression in human fibroblasts by interfering with the IFN-\(\gamma\)-induced signal transduction (Jak/Stat) pathway (Fig. 7). This study represents the first report of a mechanism for VZV-mediated disruption of IFN-\(\gamma\)-inducible MHC class II expression and the third report of a direct virus-associated alteration in Jak/Stat protein components. Significantly, these experiments revealed the inhibition of MHC class II in VZV-infected skin cells in vivo. These cells do not express MHC class II, despite the presence of inflammatory cells, suggesting that this effect may transiently protect infected cells from CD4\(^+\) T-cell immune surveillance. These findings provide another example of the diverse immunomodulatory functions that VZV and other viruses utilize to avoid immune surveillance and establish persistent infection in the host.

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FIG. 7. Schematic diagram of the sites of VZV-mediated disruption of IFN-
\(\gamma\)-induced MHC class II expression. The various proteins that are affected in VZV-infected cells are crossed out.