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Vaccinia virus (VV)1 is a complex DNA virus that replicates in the host cell cytoplasm. VV has been used as an attenuated vaccine to prevent smallpox transmission and has been proposed as a vaccine for infectious agents and tumors. Although VV is highly effective in inducing long-lasting protective immunity in a majority of healthy individuals, potentially serious complications following VV infection have limited enthusiasm for this virus as a universal vaccine reagent (1–3). VV evades the host immune system affecting both innate and adaptive immunity (4). In epithelial and fibroblast cells, VV decreases host protein synthesis by disruption of host DNA replication (5), interference with cellular RNA synthesis and processing, and promotion of RNA degradation (6, 7). Yet, not all host gene expression is blocked with VV infection, given that the virus exploits host proteins to survive and replicate. A recent analysis of dendritic cell gene expression upon exposure to

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3 Abbreviations used in this paper: VV, vaccinia virus; AEP, asparagine endopeptidase; ArAC, arabinoadenosine-5’-triphosphate; B-LCL, B lymphoblastoid cell line; Cat, cathepsin; CHX, cycloheximide; GAD, glutamate decarboxylase; HSA, human serum albumin; HSC, heat shock cognate protein; Ii, invariant chain; Leu, leupeptin; LIP, leupeptin-induced peptide; MOI, multiplicity of infection; CLIP, class II-associated invariant chain peptide.

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Vaccinia virus (VV)1 has been used as a vaccine to eradicate smallpox and as a vaccine for HIV and tumors. However, the immunoevasive properties of VV have raised safety concerns. VV infection of APCs perturbs MHC class II-mediated Ag presentation. Exposure of human B cell lines to VV induced a substantial reduction in cellular expression of the class II chaperone, invariant chain (Ii), during the late stages (i.e., 8–10 h) of infection. Yet, cell viability and surface expression of MHC class II molecules were maintained up to 24 h after exposure to virus. Reductions in Ii and class II mRNA levels were detected as early as 6 h after VV infection of APCs. To examine whether VV was acting solely to disrupt host protein synthesis, B cells were treated with an inhibitor of translation, cycloheximide (CHX). Within 1 h of B cell CHX treatment, Ii protein expression decreased coupled with a loss of class II presentation. Analysis of Ii degradation in VV- or CHX-treated cells, revealed ongoing Ii proteolysis contributing to reduced steady-state Ii levels in these APC. Yet in contrast with CHX, VV infection of APCs altered lysosomal protease expression and Ii degradation. Virus infection induced cellular cathepsin L expression while reducing the levels of other lysosomal proteases. These results demonstrate that at late stages of VV infection, reductions in cellular Ii levels coupled with changes in lysosomal protease activity, contribute in part to defects in class II presentation. The Journal of Immunology, 2009, 183: 1542–1550.

Studies have demonstrated diminished MHC-mediated Ag presentation upon APC infection by VV (9–19). An early, transient reduction in CD4+ T cell responses after VV vaccination of humans suggests diminished APC function in vivo following exposure to VV (18). Minimal to no virus infection of activated and resting T cells has been reported (20, 21). By contrast, APCs (monocytes, dendritic cells, and B cells) are highly susceptible to VV infection (11, 21, 22). VV disruption of MHC class II-restricted Ag presentation is observed with both professional and nonprofessional APC (10, 13–15). Although inhibition of host protein synthesis was postulated as one potential explanation for virus inhibition of class II presentation (13), others demonstrated sustained surface expression of class II proteins in APCs up to 24 h after VV exposure (10, 11).

MHC class II complexes consist of αβ dimers that pair with the chaperone protein invariant chain (Ii) in the endoplasmic reticulum. These αβIi complexes translocate to the Golgi and are targeted to endosomes where Ii is degraded by acidic proteases such as cathepsins (Cat) S and L (23, 24). HLA-DM releases Ii fragments from the ligand-binding groove of class II to permit binding of peptide fragments from processed Ags. Finally, these peptide-class II complexes translocate to the surface of APCs for presentation to CD4+ T cells. As a chaperone, Ii directs the folding of the class II αβ dimer and transports class II molecules to compartments rich in antigenic peptides. Thus, Ii influences class II protein function and the selection of CD4+ T cells (25–29).

In this report, significant reductions in intracellular Ii protein and mRNA levels were detected in human B-lymphoblastoid cell lines (B-LCL) at the late stages of VV infection. Yet even late in infection, low levels of new class II protein synthesis could readily be detected. Like VV, cycloheximide (CHX) also decreased Ii protein expression and class II function, whereas surface class II expression was maintained. Proteolytic processing of Ii was observed in both VV- and CHX-treated APC, contributing to diminished intracellular Ii. Yet, alterations in lysosomal protease expression...
were apparent only in VV-infected APCs. Virus infection induced host Cat L expression while diminishing the levels of several other lysosomal proteases. Thus, at late stages of B cell VV infection, reductions in intracellular Ii likely contribute to reduced APC function.

Materials and Methods

Virus and cells

VV Western Reserve strain was cultured, gradient purified, and titrated as previously described in CV-1 African green monkey kidney cells (10). CV-1 cells were cultured in DMEM (Invitrogen Life Technologies) with 10% FBS, 2 mM t-glutamine, 50 U/ml penicillin, and 50 µg/ml streptomycin. Priess, a human B-LCL-expressing class II DR4, and PriessGAD, Priess cells retrovirally transduced for constitutive expression of human glutamic acid decarboxylase (GAD), were cultured in IMDM (Invitrogen Life Technologies) with 10% heat-inactivated calf serum, 50 U/ml penicillin, and 50 µg/ml streptomycin. T cell hybridomas, 33.1 specific for GAD273-285, and 17.9 specific for human serum albumin (HSA)64–72, both presented in the context of HLA-DR4, were cultured in RPMI 1640 (Invitrogen Life Technologies) with 10% FBS, 50 U/ml penicillin, 50 µg/ml streptomycin, 2 mM t-glutamine, and 50 µM 2-ME. HT-2, a 2–dependent T cell line, was grown in RPMI 1640 with 10% FBS, 50 U/ml penicillin, 50 µg/ml streptomycin, 50 µM 2-ME, and 20% T stim (BD Biosciences). For quantification of IL-2 using HT-2 cells, T stim was omitted from the culture medium.

Peptide and protein Ags

Peptides were manufactured using F-moc technology with HPLC analysis confirming purity of >90% (10, 30). HSA protein (peptide-free preparation) was obtained from Sigma-Aldrich.

Ag presentation assays

APCs were cultured in the absence or presence of VV (multiplicity of infection (MOI) 10) for 2–14 h. APCs were then washed and fixed with 0.5% paraformaldehyde for 10 min at 4°C. Cells were coinoculated with fixed, and cocultured with HSA-specific T cells. To determine the effects of CHX on endogenous Ag presentation, APCs were treated with 1 µM CHX for 0–14 h before fixation and T cell coculture. To investigate the effect of CHX on endogenous GAD Ag presentation, PriessGAD were treated with VV ± the viral DNA polymerase inhibitor, arabinosylcytosine (AraC, 50 µg/ml; Sigma-Aldrich) for 0–14 h before fixation and coculturing with GAD-specific T cells. To compare the effects of CHX and VV on exogenous Ag presentation, APCs were treated with 1 µM HSA Ag ± 10 µg/ml CHX for 6 h or VV (MOI 10) for 14 h, then fixed, and cocultured with HSA-specific T cells. To determine the effects of CHX or VV on peptide presentation, Priess cells were coinoculated with 1 µM HSA64–72 or GAD273-285 peptide ± CHX or VV before addition of T cells. APC viability was assessed by trypan blue exclusion during exposure to virus or CHX, and conditions optimized to ensure little to no decrease in cell viability during these treatments. T cell activation was quantified by IL-2 production using an HT-2 bioassay (31). All assays were performed in triplicate, and the relative mean proliferation and SDs are expressed as cpm.

Western blotting

Cells treated with VV (MOI 10) ± an optimal concentration of AraC (10 µM) or with CHX (10 µg/ml) alone for different times were harvested and lysed in buffer (10 mM Trizma base, 150 mM NaCl, and 1% Triton X-100 with 1% protease inhibitors N-tosyl-lysylchloromethylketone and PMSF). Cell lysates were centrifuged to remove nuclei before the addition of SDS sample buffer. Samples of 40 or 400 µg (the latter for Cat analyses only) of total cell protein were fractionated by SDS-PAGE followed by transfer to nitrocellulose membranes. Membranes were probed for: DRα, DRβ1 dimer using mAb D6A.147; DRβ2 dimer using mAb L243; MHC class I H chain using mAb 3B10.7; GAD using a polyclonal Ab (Sigma-Aldrich); Ii using mAb Pin 1.1; Cat S using Cat S Ab (Biovision); and Cat L using the mAb CPLH 36.1 (32). Cellular actin was detected using mAb Pan Actin Ab-5 (Lab Vision), and GAPDH using GAPDH mAb (Millipore). VV Ags were detected using a rabbit antisera recognizing abundant virion products (24), as well as rabbit Abs specific for epitopes within the D8 (32 kDa) and H3 (35 kDa) late viral Ags. Virion Ags D8 and H3 were similarly detected using these Abs. Densitometric analysis of Western blots was conducted with software ImageJ (National Institutes of Health Web site) using cellular actin or GAPDH expression as a relative internal standard.

To monitor the effects of VV on Ii maturation and processing, PriessGAD cells were cultured with VV (MOI 10) for 2 h to permit infection, followed by incubation with protease inhibitors for an additional 12 h for a total infection time of 14 h before analysis by Western blotting. Inhibitors used here were 0.5 mM leupeptin (Leu; Sigma-Aldrich); 0.5 mM E64 (Sigma-Aldrich); and 1 mg/ml asparagus endopeptidase (AEP) inhibitor peptide AENK (33, 34). To study the effects of CHX on Ii processing, PriessGAD cells were pretreated with Leu for 1 h; then CHX (10 µg/ml) was added to the medium for an additional 4 h, followed by Western blotting and densitometry. Cell viability was >80% after each inhibitor treatment.

f15S]Methionine incorporation and pulse-chase assays

To assess the effects of VV on host protein synthesis, PriessGAD cells (1 × 105/sample) were infected with VV (MOI 10) for 0, 2, 6, and 14 h before addition of 0.5 mCi of [35S]methionine (MP Biomedicals) in cold-free RPMI 1640 for 1 h. Cells were harvested, lysed, and immunoprecipitated with mAb Pin 1.1 for Ii and DA6.147 for DR. The precipitated samples were resolved on the SDS-PAGE followed by Coomassie blue gel staining and autoradiography. Coomassie blue gel staining confirmed equal sample loading. For pulse-chase assays, PriessGAD cells were starved in methionine-free RPMI 1640 for 1 h and labeled with [35S]methionine for 1 h. Samples were chased in medium without unlabeled methionine ± VV (MOI 10) and/or E64 (500 µM) for 0, 2, 6, and 14 h. Samples were immunoprecipitated and analyzed by SDS-PAGE and autoradiography.

Semiquantitative RT-PCR and quantitative real-time PCR

PriessGAD cells were treated ± VV (MOI 10) for up to 14 h. Total cellular RNA was extracted with an RNeasy Mini Kit (Qiagen). cDNA was generated from RNA using an Advantage RT for PCR kit (BD Biosciences). Primers for PCR amplification were designed using custom Primers-OligoPerfect Design software (Invitrogen). The primers used were: human Ii, 5’-GGCTTGGCGGAAGATCGAG-3’ (sense) and 5’-GCCATACTTTGTTGCATCT-3’ (antisense); DRα, 5’-CAAGAAGAGGACAGGTCTG-3’ (sense) and 5’-AGCATAAATCTACCCATGGT-3’ (antisense); Cat G3, 5’-GGATCACGACTGTCATCTC-3’ (sense) and 5’-CCAGCTTTCCTGTITTATGCAC-3’ (antisense); Cat G5, 5’-GGCTATCTGGTGGTAAGCCT-3’ (sense) and 5’-AGATTCTGATCCAGTTG-3’ (antisense); Cat D, 5’-AGCCTGGTGGACAGAACATC-3’ (sense) and 5’-CTCTGGGACAGCTTGTAGC-3’ (antisense); Cat L, 5’-TGGTGGTCTTCTGGGGTCTT-3’ (sense) and 5’-CAGGCTTCTTATCCTGGA-3’ (antisense); Cat S, 5’-AGAAATCTTGCGACACACC-3’ (sense) and 5’-CCATCTCTTGAGTACGTCCTC-3’ (antisense); Cat B, 5’-GCTATCTGGTGGTAAGCCT-3’ (sense) and 5’-CATTGTCACCCCACTGTCAGT-3’ (antisense); and Cat E3, 5’-AGGTTGTTTTCTGACGGG-3’ (sense) and 5’-AGCTGAGATTGTGTGTGA-3’ (antisense). To detect Ii, DRα, Cat D (32 cycles); Cat L (40 cycles); and Cat B (60 cycles). The cycling parameters used were: 95°C, 15 s; 50°C, 30 s; and 68°C, 1 min. PCR products were electrophoresed on 1.5% agarose gels, stained with SYBR Safe DNA gel stain (Invitrogen), and detected with UV transillumination using the BioDoc XRS (Bio-Rad). mRNAs for early viral gene products E3L and late gene product D8L were monitored as evidence of VV infection. Host cell HSC70 was used as a control for sample loading. Similar procedures were used to detect relative mRNA abundance after CHX treatment. mRNA expression levels in CHX-treated samples were evaluated and averaged from three independent experiments with the SD indicated.

To detect Ii, DRα, and HSC70 mRNA levels by quantitative real-time PCR, mRNA isolation, and cDNA synthesis were performed as above. TaqMan Gene Expression Assays with specific primers and probes (Hs00269961_m1 for Ii; Hs00195751_m1 for DRα; and Hs00852842_gH for HSC70) were incubated with TaqMan Fast Universal PCR Master Mix and cDNA templates. Samples were amplified 40 cycles with a 7500 Fast Real-Time PCR System (Applied Biosystems) with the following parameters: 95°C, 15 s; and 60°C, 1 min. HSC70 was used as the endogenous reference, and 0.1 relative quantification of Ii or DRα mRNA was used as the calibrator.
Flow cytometry

For surface class II-associated invariant chain peptide (CLIP) staining, cells were incubated with FITC-anti-human CLIP Ab or corresponding isotype control Ab (BD Biosciences) on ice for 30 min followed by washing and fixation with 0.5% paraformaldehyde. Samples were gated on live cells and analyzed by flow cytometry using CellQuest software (BD Biosciences).

DNA sequencing

To confirm the PCR product amplified using primers specific for human Cat L, PriessGAD cells were cultured ± VV (MOI 10) for 14 h followed by mRNA isolation and cDNA synthesis. After amplification of cellular mRNA using the Cat L primers, the reaction products from each sample were separated on a 1% agarose gel, and the amplified cDNA bands were excised and extracted using a QIAquick gel extraction kit (Qiagen). The cDNAs were sequenced by the DNA Sequencing Core Facility at Indiana University School of Medicine using a PerkinElmer/Applied Biosystems 3100 Genetic Analyzer and Big Dye Terminator chemistry version 3.1. Cat L cDNA amplified from uninfected PriessGAD cells was sequenced and compared with this gene amplified from VV-infected PriessGAD cells using ClustalW software from the European Bioinformatics Institute. To confirm the identity of these amplified cDNA sequences as human Cat L, nucleotide-nucleotide BLAST software from the National Center for Biotechnology Information was used.

Results

MHC class II function and protein expression during VV infection of APC

VV inhibits MHC class II-mediated presentation of Ags and peptides by APC (10). Viral infection of PriessGAD cells reduced MHC class II presentation of an endogenous Ag, GAD dependent on viral MOI and the duration of infection (Fig. 1, A and B). The inhibitory effects associated with virus infection progressed over time without diminishing cell viability.

The inhibitory effect of VV on Ag presentation was observed initially at early stages of APC infection (≤ 2 h), and prior studies demonstrated that this effect was independent of VV replication (10). VV blocks host protein synthesis shortly after entry into fibroblasts (35). Thus, it remained possible that VV disruption of Ag presentation was linked to changes in the expression of MHC class II proteins or other components of class II pathway during virus infection. Protein levels for both MHC class I and MHC class II molecules were assessed during 24 h of VV infection. VV infection did not significantly diminish steady state expression of MHC class II DRα monomers or DRαβ dimer levels up to 24 h (Fig. 1, C and D). Also, VV infection of B-LCL did not influence the level of endogenous GAD Ag, MHC class I H chain and cellular actin expression in B-LCL. As expected, the expression of viral late membrane D8 and H3 Ags increased as infection progressed.

VV infection decreased Ii expression in B cells at both the protein and mRNA levels

Although expression of MHC class II proteins remained constant, steady-state levels of cellular Ii decreased in a time dependent manner upon VV infection of B-LCL (Fig. 2A). Ii protein levels were slightly decreased at 2 h of infection with a more pronounced drop between 8 and 12 h of infection. Densitometric quantification revealed a 15% reduction in cellular Ii abundance at 2 h, with an 80% decrease in Ii levels at 12–14 h of VV infection. To determine whether the reduction in Ii expression occurred at the level of protein synthesis, biosynthetic radiolabeling was performed. PriessGAD cells were infected with VV for up to 14 h with radiolabeled methionine added at different times before immunoprecipitation of Ii and class II proteins. At 6 h, VV infection decreased Ii synthesis by 44%, yet DRα synthesis was preserved (Fig. 2B). Even at 14 h, new synthesis of class II was detected but little if any new Ii protein was synthesized by infected cells. To further establish that VV inhibits Ii synthesis, the relative abundance of Ii mRNA was measured using semiquantitative RT-PCR analysis. Ii mRNA levels progressively decreased after VV infection, temporally preceding the drop in Ii protein expression (Fig. 2C). Host HSC70 mRNA levels, used as a loading control, did not change up to 14 h after VV infection. To more accurately monitor alterations
in Ii mRNA levels after VV infection, real-time PCR was performed using HSC70 as the endogenous reference. Quantification of Ii mRNA during VV infection revealed a 65% decrease after 6 h and an 80% drop after 10 h (Fig. 2D). These results indicate diminished Ii synthesis contributes to the reduced steady-state levels of Ii protein during VV infection. To further investigate the effect of virus on Ii expression and GAD presentation, B-LCL were treated with VV/H11006/araC for 14 h. AraC is a virus-specific DNA polymerase inhibitor that blocks replication and translation of late viral gene transcripts (36). As shown in Fig. 2E, decreased Ii expression and GAD presentation were observed at 14 h of VV infection but could be partially prevented by AraC treatment. AraC alone did not affect cellular Ii expression or GAD presentation. AraC treatment did not alter the effects of virus on Ag presentation at 6 h. Similar results were obtained at higher concentrations of AraC. Expression of viral late Ags D8 and H3 were reduced in cells exposed to VV and AraC. Studies using UV-treated virus also indicated virus replication was not essential for the observed loss of class II function (10). Thus, studies with AraC suggest that at late stages of virus infection, loss of cellular Ii contributes in part to decreased class II function.

**Inhibition of host protein synthesis with CHX rapidly reduced cellular Ii levels with no immediate change in MHC class II steady-state protein expression**

CHX, a protein synthesis inhibitor, has been used to investigate the effects of disrupting host protein synthesis on the class II pathway as well as to study changes in host protein expression with poxvirus infection (29, 37). CHX interrupts both the initiation and extension of de novo protein synthesis by acting at the translation level (38, 39). Here, CHX efficiently decreased B cell protein synthesis with a minimal loss of cell viability after 6 h, as determined by monitoring radiolabeled amino acid incorporation into host proteins (data not shown). Cell lysates from CHX-treated B-LCL were assessed for class II DRα, DRβ, class I, GAD, and Ii protein expression (Fig. 3, A–C). Similar to VV infection, Ii protein levels were significantly diminished with CHX treatment, yet steady-state levels of other class II pathway components and endogenous GAD Ag expression were...
relatively unaffected. CHX rapidly diminished cellular Ii protein expression in a time-dependent manner, and this loss of Ii was significantly faster than observed with VV (Figs. 2A and 3C). At longer times of treatment, CHX induced cytopathic effects; hence, studies were limited to 6 h. Consistent with CHX acting at the translational level, mRNA for both Ii and DRα remained constant after CHX treatment of B-LCL for 6 h (Fig. 3D).

**Inhibition of MHC class II presentation by CHX treatment of B-LCL**

In contrast with VV, published studies had demonstrated reductions in class II Ag but not peptide presentation upon B cell exposure to CHX (29, 40). Studies were performed to determine whether similar changes in class II function were observed using CHX concentrations that promote the loss of cellular Ii. As shown in Fig. 4A, CHX treatment inhibited endogenous GAD Ag presentation in a time-dependent manner. The reduction in endogenous GAD Ag presentation correlated temporally with the loss of Ii protein in CHX-treated B-LCL (Fig. 3C). As shown in Fig. 4B, exposure of B-LCL to CHX significantly reduced exogenous Ag presentation comparable with VV inhibition. The effect of CHX or VV on peptide presentation by B cells was tested (Fig. 4, C and D). CHX treatment of B-LCL inhibited HSA peptide presentation much like VV infection (Fig. 4C). However, CHX consistently disrupted GAD peptide presentation less severely than VV infection (Fig. 4D). HSA peptide must be processed by APCs in early endosomes before presentation by class II molecules (30). By contrast, the GAD epitope can directly bind to surface class II without processing (41). Previous studies also suggested minimal inhibition of surface peptide presentation by CHX (29, 40). Thus, although both CHX and VV disrupt class II Ag presentation by B cells, differences in the ability of each agent to perturb peptide presentation were observed. Class II presentation of protein Ags was typically more sensitive to inhibition by VV or CHX, when compared with class II display of exogenously added peptides and these agents.

**VV infection influences Ii degradation**

Although protein synthesis inhibition is likely the predominant mechanism leading to diminished Ii protein expression in cells exposed to VV or CHX, the proteolytic cleavage of this chaperone within endosomal compartments may also influence Ii steady-state abundance. Within these organelles, Ii is proteolytically cleaved to leupeptin-induced peptide (LIP), small LIP, and then CLIP, a terminal product of Ii degradation. Disruption of Ii proteolysis can perturb class II maturation and function. It has been established that some viral proteins, such as HIV-2 and SIV virion-associated protein Vpx, reduce cellular Ii expression by promoting Ii degradation (42).

To test whether VV infection alters Ii degradation, B-LCL were exposed to VV in the presence of Leu. Leu inhibits cysteine proteases such as Cat B, L, and S reversibly, perturbing Ii degradation and resulting in cellular Ii and LIP accumulation (34, 43). PriessGAD cells were pretreated ± VV, followed by culture with Leu and analysis of cellular Ii levels by Western blotting. In Fig. 5A, VV infection decreased cellular Ii levels by 79% compared with controls at 14 h. Leu treatment of uninfected APCs (1 × Leu) resulted in Ii and LIP accumulation. Cellular VV infection coupled with Leu treatment decreased Ii levels by ~60%, compared with Leu treatment only. Similar levels of viral infection were found in cells ± Leu as assessed by the relative abundance of VV proteins. These results indicate Ii proteolysis continued during VV infection, coupled with diminished Ii synthesis. Studies suggest that AEP can catalyze the earliest steps in Ii proteolysis (33). The addition of an AEP inhibitor AENK to APCs resulted in Ii accumulation, consistent with a role for AEP in Ii proteolysis. AENK treatment did not alter virus infection, but similar to Leu it preserved Ii levels in cells, consistent with AEP functioning in Ii proteolysis during VV infection. Analysis of the later steps in Ii
processing revealed low amounts of LIP at 14 h in VV plus Leu-treated cells compared with uninfected cells plus Leu (Fig. 5, A and C). To suppress cellular Ii proteolysis during VV infection, repeated additions of Leu or an irreversible cysteine protease inhibitor, E64, were required, leading to greater LIP and Ii accumulation (Fig. 5, A and C). These results suggested enhanced Ii processing at the late stages of VV infection. Consistent with this result, flow cytometry revealed slightly elevated levels of the Ii fragment CLIP at the late stages of APC VV infection (Fig. 5E).

Thus, AEP- and Leu-sensitive proteases appear to play important roles in Ii processing during VV infection. In testing whether inhibition of cellular protein synthesis directly perturbs Ii processing,
cathepsins tested, Cat S plays a key role in the later stages of Ii cleavage in B cells (24, 45). Thus, a reduction in Cat S mRNA may lead to aberrant Ii processing. Viral infection also led to a decrease in message for Cat B and Cat D. These Cats function in processing Ag within APC (44, 46). Cat L mRNA increased in a time-dependent manner during VV infection of B cells (Fig. 6A). Cat L mediates Ii processing in cortithymic epithelial cells to regulate positive selection (23, 47). A recent study demonstrated Cat L expression in human B cell lines (24, 48–53). To further confirm Cat L expression in cells ± VV infection, the detected mRNA was amplified, converted to cDNA, and sequenced. The more abundant amplified sequence from VV-infected cells as well as the cDNA sequenced in control cells were both identified as human Cat L. To test whether altered cathepsin expression was observed during VV infection, Western blotting analysis was conducted with cathepsin-specific Abs. Corresponding with mRNA levels, VV infection of APCs reduced Cat S protein abundance while enhancing Cat L protein levels (Fig. 6B). Reduced Cat S levels may account for reduced or slow Ii degradation at the transition from early to late stages of VV infection. Later, induction of cellular Cat L likely substitutes for Cat S to promote enhanced Ii proteolysis in virus-infected B cells (Fig. 5C). By contrast, CHX treatment did not alter the abundance of these two cathepsins in APC after short incubations.

**Discussion**

Although the disruption of MHC class II Ag presentation by VV infection has been well established (10), the precise events leading to this inhibition remain poorly defined. Multiple steps within the class II pathway may be perturbed by VV during the transition from early to late stages of infection. Yet few studies have addressed temporal changes within APC during VV infection that might influence class II presentation. VV infection of APCs reduces peptide binding to class II molecules, suggesting destabilization of class II and consequently reduced presentation to CD4+ T cells (10). In the current study, a time-dependent loss of Ii protein and mRNA was observed during the late stages of VV infection of human B cell lines (Fig. 2). Ii is important for class II αβ maturation and the presentation of endogenous and exogenous Ag as well as peptides. Although the virus-induced loss of Ii was gradual, by 14 h of infection little if any new Ii synthesis was detected (Fig. 2). Yet, new synthesis of class II subunits could still be observed at this time. Immunoblotting also revealed a significant depletion of the total pool of cellular Ii at this late stage. Similar substantial losses in cellular Ii induced either by antisense oligodeoxynucleotides or gene deletion reduced class II presentation of select Ags by 60–90%, whereas enhancement of Ii expression by either transfection or knock-in increased Ag presentation (25, 26). Thus, reductions in cellular Ii during VV infection likely contribute to the loss of class II function.

To study the mechanisms responsible for Ii loss during APC infection by VV, two pathways were explored: inhibition of Ii synthesis and enhanced Ii degradation. Expression of Ii mRNA and new protein synthesis decreased during infection, contributing to the decrease in steady-state cellular Ii protein between 6 and 14 h (Fig. 2). A similar reduction in Ii mRNA was observed upon VV infection of human PBMCs (data not shown). In B cells, these changes were observed during the late stages of VV infection and paralleled viral late protein expression (Figs. 1, 2, and 6). Class II mRNA levels also decreased at this stage of infection, yet new synthesis of class II subunits persisted at low levels at 14 h and surface class II protein expression was stable up to 24 h. Class II complexes in human B cells display half-lives of ~36 h, likely
accounting for the preservation of surface class II (54). Aggregation of some murine class II alleles is observed in II-deficient mice (28). Yet during VV infection, such high-molecular-mass aggregates of class II DR4 were not observed. Studies with II-deficient mice also suggest that HLA-DM expression is reduced (28, 55). However, no changes in HLA-DM levels were found in human B cell lines infected with VV (data not shown). Thus, virus-induced changes in the class II pathway in these human B cells do not precisely mirror the effects of II loss observed with some II-deficient mouse strains (28, 55). Analysis of intracellular αβII complexes during VV infection of B cells did reveal reduced levels of II coprecipitating with αβ after 10–12 h (data not shown). Reduced levels of αβII complexes were also detected in murine cells treated with CHX (29). In the current study, CHX treatment of APC rapidly led to reduced cellular II expression and defects in both Ag and peptide presentation by human APCs.

The effects of VV infection on the class II pathway go beyond altering host protein synthesis; changes in Cat expression were also detected during virus infection. Expression of Cat S, which is required for terminal II proteolysis was greatly diminished during virus infection. Pulse-chase studies revealed II proteolysis was initially slowed during VV infection (Fig. 5C), consistent with an observed decrease in Cat S levels. The half-life of II in human B cell lines is typically 2–2.5 h (34, 56); yet late in VV infection, II degradation appeared to be enhanced, possibly due to changes in the protease content of the cell. VV infection of human B cells resulted in induction of Cat L mRNA and increased expression of the active form of Cat L protease. Remarkably, mRNA expression of other lysosomal cathepsins, Cat S, B, and D decreased with virus infection, suggesting cellular proteolytic processing is altered. Cat S controls LIP processing in many APCs. Yet in thymic epithelial cells Cat L substitutes for Cat S to proteolyze LIP and promote class II maturation. Why VV infection promotes increased Cat L expression is unclear. Viral induction of host Cat L may facilitate infection, as has been observed for mouse hepatitis virus type 2 and acute respiratory syndrome coronavirus (57, 58). Or VV could use Cat L to promote extracellular matrix degradation, leading to tumors (59, 60). Alternatively, elevated Cat L may rep-
class II-associated invariant chain processing and peptide loading. Immunology 4: 357–366.


