Inhibition of MHC class I-restricted antigen presentation by γ2-herpesviruses

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The γ-herpesviruses, in contrast to the α- and β-herpesviruses, are not known to inhibit antigen presentation to $CD8^+$ cytotoxic T lymphocytes (CTLs) during lytic cycle replication. However, murine γ-herpesvirus 68 causes a chronic lytic infection in $CD4^+$ T cell-deficient mice despite the persistence of a substantial CTL response, suggesting that CTL evasion occurs. Here we show that, distinct from host protein synthesis shutoff, γ-herpesvirus 68 down-regulates surface MHC class I expression on lytically infected fibroblasts and inhibits their recognition by antigen-specific CTLs. The viral K3 gene, encoding a zinc-finger-containing protein, dramatically reduced the half-life of nascent class I molecules and the level of surface MHC class I expression and was by itself sufficient to block antigen presentation. The homologous K3 and K5 genes of the related Kaposi's sarcoma-associated virus also inhibited antigen presentation and decreased cell surface expression of HLA class I antigens. Thus it appears that an immune evasion strategy shared by at least two γ-herpesviruses allows continued lytic infection in the face of strong CTL immunity.

The herpesviruses persist and periodically reactivate to complete lytic cycle replication despite established host immunity. Because $CD8^+$ cytotoxic T lymphocytes (CTLs) can potentially eliminate any MHC class I-positive cell expressing viral antigens, this implies some form of CTL evasion. The herpes simplex virus ICP-47 inhibits peptide transport into the endoplasmic reticulum (1, 2), whereas various cytomegalovirus proteins either inhibit the assembly of MHC class I/peptide complexes or promote their degradation (3, 4). However, a CTL evasion mechanism effective during the lytic cycle has not been identified for γ-herpesviruses. The Epstein–Barr virus IL-10 homolog can inhibit peptide transport (5) but has little or no adverse effect on CTL recognition (6, 7), and whether Epstein–Barr virus subverts the presentation of lytic phase epitopes *in vivo* is unknown (8).

The murine γ-herpesvirus 68 (γHV-68) is a γ2-herpesvirus of small rodents that is closely related to the Kaposi's sarcoma-associated virus (KSHV) in humans (9, 10). Infection of conventional mice with γHV-68 causes an infectious mononucleosis-like illness (11–13) and elicits a strong CTL response (14). Although $CD8^+$ T cells help to limit the acute epithelial infection, their absence is generally tolerated (15). In contrast, a lack of $CD4^+$ T cells leads to a lethal wasting disease associated with chronic lytic viral replication (16). This does not reflect CTL exhaustion in $CD4^+$ T cell-deficient mice (17); indeed, there is some increase in their virus-specific $CD8^+$ T cell numbers as the antigen load rises (17), and even massively boosting the CD8 response by postexposure, epitope-specific vaccination does not improve survival (18). Clearly CTLs can respond to some γHV-68-infected cells but cannot fully control lytic cycle replication. The viral M3 protein is known to bind a broad range of chemokines (19) and could potentially diminish CTL efficacy. However, *in vivo* analysis has shown little effect of a lack of M3 on the immune control of epithelial infection (unpublished data).

Bulk-cultured, γHV-68-specific CTLs kill virus-infected target cells, but with rather low efficiency (14, 20). Using CTL clones specific for immunodominant lytic cycle epitopes, we found little or no recognition of virus-infected fibroblasts (14). This finding suggested that γHV-68 interferes with CTL recognition, but the result was difficult to interpret, because γHV-68 seems to present different lytic cycle epitopes in different situations (14, 17), and even epitopes that are immunodominant *in vivo* might not be presented *in vitro*. The present analysis shows that γHV-68 does indeed inhibit MHC class I-restricted antigen presentation and identifies a gene, relatively conserved among γ2-herpesviruses, that can reproduce this effect.

Materials and Methods

Cell Lines and Viruses. Cell lines were obtained from the American Type Culture Collection unless stated otherwise, and were grown in DMEM supplemented with antibiotics and 10% FCS. Murine embryonic fibroblasts (MEFs) were harvested from 13- to 14-day embryos and used at passage 3–6. For infection, cells were exposed to γHV-68 at 10 plaque-forming units (pfu) per cell for 1 h.

Plasmids and Retroviruses. Retroviral vectors were made by calcium phosphate transfection of 293T cells with the packaging plasmid pEQPAM3 (21), plus the relevant gene cloned into the *Eco*RI/*Xho*I sites of pMSCV-GFP (22) or pMSCV-NEO. In pMSCV-GFP, green fluorescent protein (GFP) is translated from an internal ribosome entry site downstream of the *Xho*I site. To make pMSCV-NEO, neomycin phosphotransferase was substituted for GFP by replacing an *Apa*I/*Age*I fragment of pMSCV-GFP with an *Apa*I/*Xba*I fragment of pMENA (23). All cloned genes were amplified by PCR, including *Eco*RI and *Xho*I or *Sal*I restriction sites in the respective 5′ and 3′ oligonucleotide primers. Where indicated, genes were further subcloned into pCDNA3 (Invitrogen). The influenza nucleoprotein gene, the $H\text{-}2K^b$ heavy chain, and ovalbumin were amplified from cDNA derived from influenza A/WSN-infected Madin–Darby canine kidney cells, C57BL/6J mouse lymph node, and chicken oviduct, respectively. γHV-68 ORF 50, ORF 61, and ORF K3 were amplified from virion DNA; the KSHV ORF K3 and ORF K5, from BC-3 cell DNA (ATCC CRL-2277); and the m152 gene of murine cytomegalovirus, from viral DNA. The polyepitope M-TSINFVKI-ASNENMETM-SIINFEKL was synthesized as

Abbreviations: CTL, $CD8^+$ cytotoxic T lymphocytes; γHV-68, murine γ-herpesvirus 68; GFP, green fluorescent protein; KSHV, Kaposi's sarcoma-associated herpesvirus; MEF, murine embryonic fibroblast; pfu, plaque-forming units.

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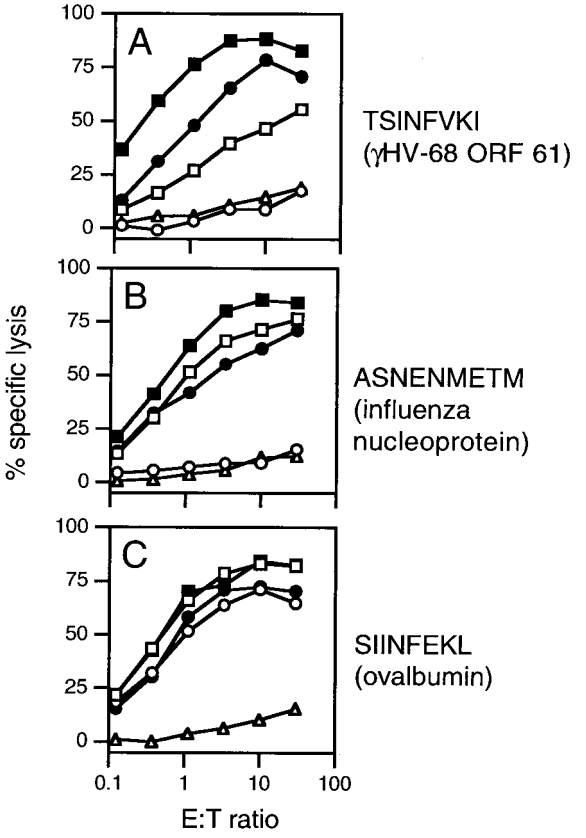

Fig. 1. Inhibition of CTL-induced ^{51}Cr release by γHV-68. MEF-1 cells transduced with retrovirus from pMSCV-GFP derivatives were uninfected (□, ■) or infected overnight with γHV-68 (○, ●) before being used as targets in 6-h ^{51}Cr release assays. E:T, effector-to-target cell ratio. Target cells transduced with pMSCV-GFP alone are shown as triangles. For filled symbols, the peptide cognate for the particular CTL line was added (1 μM) at the time of infection. The pMSCV-GFP derivatives were pMSCV-γHV-68 ORF 61 (amino acids 1–740)-GFP (*A*), pMSCV-influenza nucleoprotein (amino acids 1–403)-GFP (*B*), and pMSCV-ovalbumin (amino acids 1–325)-GFP (*C*). The effectors used were CTL lines specific for the TSINFVKI epitope of the ORF 61 (*A*), the ASNENMETM epitope of the influenza nucleoprotein (B), and the SIINFEKL epitope of ovalbumin (*C*).

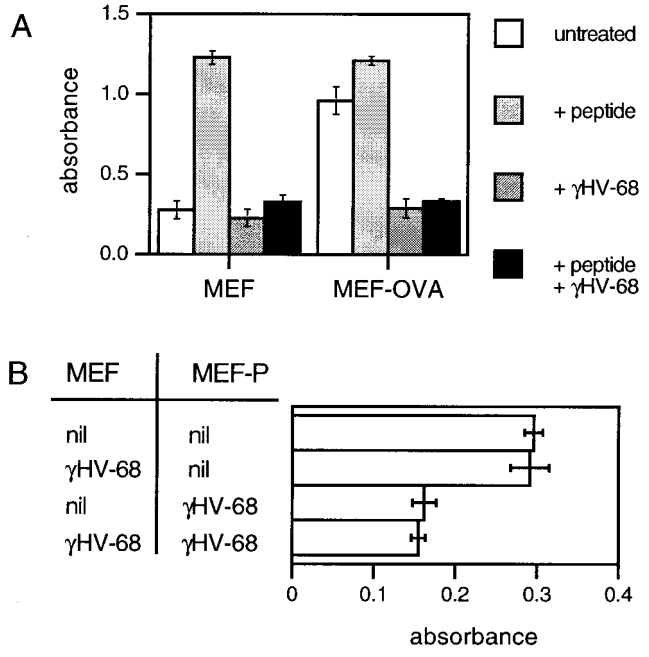

Fig. 2. Inhibition of T cell hybridoma recognition by γHV-68. (*A*) MEF-1 cells transduced either with pMSCV-ovalbumin-GFP retrovirus (MEF-OVA) or with pMSCV-GFP alone (MEF) were infected or not with γHV-68 (10 pfu per cell) and exposed or not to 1 μM SIINFEKL peptide as indicated. Six hours later, the MEF-1 cells were washed and mixed with B3Z hybridoma cells that recognize the SIINFEKL epitope of ovalbumin. β-Galactosidase production by B3Z in response to SIINFEKL presentation was then assayed with *o*-nitrophenyl galactopyranoside. (*B*) MEF-1 cells transduced with pMSCV-M-TSINFVKI-ASNENMETM-SIINFEKL-GFP polytope retrovirus (MEF-P) or with pMSCV-GFP alone (MEF) were infected or not with γHV-68 (10 pfu per cell). Six hours later, the MEF and MEF-P cells were washed three times and mixed in a 1:1 ratio as indicated before overnight culture with B3Z cells and assay for β-galactosidase production.

complementary oligonucleotides (Life Technologies, Grand Island, NY). Retroviral supernatants were harvested 48 or 72 h after transfection, filtered (0.45-μm pore diameter), and used to transduce cells in the presence of 10 μg/ml Sequa-brene (Sigma–Aldrich). Cells transduced with pMSCV-NEO derivatives were selected by culture in 500 μg/ml G418.

Bulk Culture Restimulation and Cytotoxicity Assay. CTL lines specific for the H-2K^b-restricted TSINFVKI epitope of γHV-68 were derived from γHV-68-infected C57BL/6J mice as described previously (14). Those specific for ASNENMETM (influenza nucleoprotein, H-2D^b-restricted) or SIINFEKL (ovalbumin, H-2K^b-restricted) were derived from mice infected with an influenza A/WSN recombinant expressing the SIINFEKL epitope in the neuraminidase stalk. CTL lines were used after 5–10 restimulations *in vitro*, when essentially 100% of the cells were CD8$^+$ and epitope-specific (data not shown). Cytotoxicity was measured by a 5-h ^{51}Cr release assay as described previously (14).

T Cell Hybridoma Assays. The SIINFEKL-specific, H-2K^b-restricted hybridoma B3Z (24) was cultured overnight (1.5×10^5 cells per well) with MEF-1 derivatives (5×10^4 cells per well) in 96-well plates or (1.5×10^6 cells per well) with transfected L-929-H-2K^b cells (5×10^5 cells per well) in 24-well plates. L929-H-2K^b cells were transfected using Fugene-6 (Roche Diagnostics) according to the manufacturer's instructions. B3Z cells were added 48 h after transfection. After overnight culture, the B3Z cells were washed and lysed in PBS/0.5% Nonidet P-40/5 mM $MgCl_2$/5 μM *o*-nitrophenyl galactopyranoside. The lysates were assayed 2–3 h later for absorbance at 414 nm.

Flow Cytometry. Cells were washed in ice-cold PBS/BSA (0.1%)/azide (0.01%) and stained for H-2K^b (Y3), H-2D^b (28.14.8), H-2K^d (SF-1.1.1), or HLA-ABC (W6/32), followed by rabbit anti-mouse-IgG-fluorescein isothiocyanate (Dako) or goat anti-mouse-IgG-phycoerythrin (Sigma–Aldrich). They were then washed once after a 30-min incubation on ice in each case, fixed in 1% paraformaldehyde, and acquired on a FACSort (Becton Dickinson), using LYSYS II. Data were analyzed with FCSPRESS 1.0J software (www.fcspress.com).

Analysis of Host Protein Synthesis. Cells, with or without prior γHV-68 infection (10 pfu per cell), were incubated for 1 h in methionine-free medium, pulsed for 15–60 min with [^{35}S]methionine (100–200 μCi/ml; Amersham Pharmacia), chased where indicated with an excess of unlabeled methionine, washed in PBS, and lysed for 30 min on ice in 150 mM NaCl/50 mM Tris·HCl (pH 7.2), with 1% Triton X-100/5 mM iodoacetamide/2 mM PMSF. Nuclei and cell debris were pelleted by centrifugation (10,000 × *g*, 15 min). The extent of host protein synthesis shut-off was determined by SDS/PAGE analysis of the

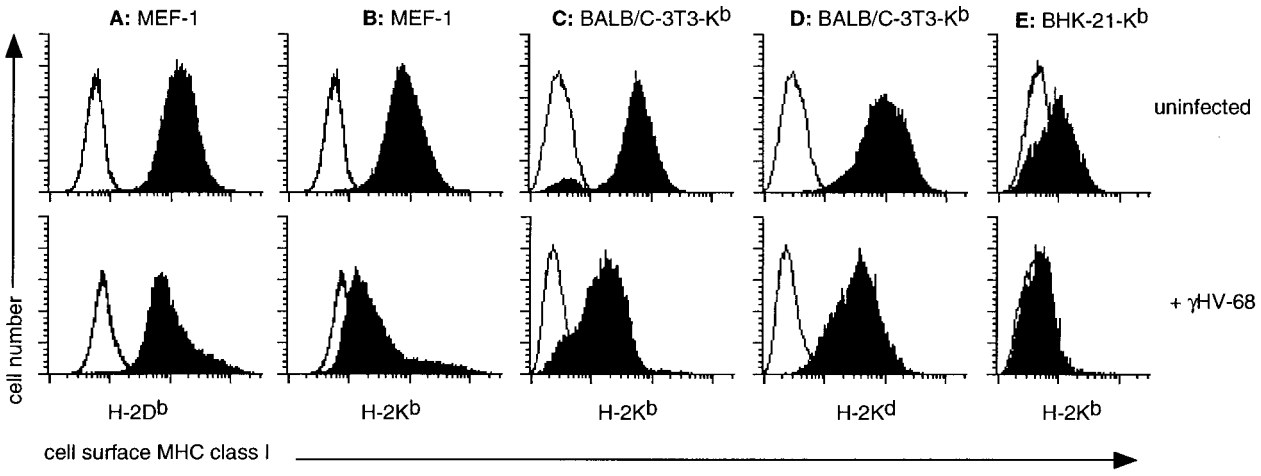

Fig. 3. Inhibition of MHC class I expression by γHV-68. To generate BALB/c-3T3-K^b cells, BALB/c-3T3 cells were transduced with retrovirus derived from pMSCV-H-2K^b-NEO. To generate BHK-21-K^b cells, equivalent retrovirus was used to infect the GP + E86 retroviral packaging line, and tunicamycin-treated (26) BHK-21 cells were then cultured with supernatant from the transduced packaging line. After selection, all transduced cells were infected with γHV-68 (10 pfu per cell) or left uninfected and then stained for surface MHC class I expression (filled histograms) or with secondary antibody alone (open histograms) after overnight culture.

supernatant. To analyze MHC class I metabolism, H-2D^b molecules were immunoprecipitated from labeled cell extracts with 28.14.8 antibody and staphylococcal protein A-Sepharose. Pulse–chase analysis, immunoprecipitations, and endoglycosidase H digestion were performed as described previously (25).

Results

Inhibition of Cytotoxicity by γHV-68 Infection. We first ensured stable expression of known CTL peptide epitopes by retroviral transduction of H-2^b MEF-1 cells with the gene for ovalbumin (SIINFEKL epitope), the influenza virus nucleoprotein (ASNENMETM), or the γHV-68 ORF 61 (TSINFVKI). These MEF-1 derivatives, either left uninfected or infected overnight with γHV-68, were used as targets for peptide-specific CTL lines (Fig. 1). Recognition of the retrovirus-expressed TSINFVKI and ASNENMETM epitopes was blocked by γHV-68 infection (Fig. 1*A* and *B*), even though TSINFVKI is itself processed (14) from the γHV-68 ribonucleotide reductase (ORF 61). In contrast, the presentation of retrovirus-expressed SIINFEKL by γHV-68-infected cells was still sufficient to trigger lytic activity (Fig. 1*C*), and every epitope was adequately presented from a large dose of exogenous peptide. Thus it appeared that unless epitope expression was optimal, γHV-68 infection inhibited lysis.

Inhibition of T Cell Hybridoma Recognition by γHV-68 Infection. The SIINFEKL-specific B3Z T cell hybridoma (24) was also used, to exclude the possibility that an increased resistance to lysis of γHV-68-infected cells rather than defective antigen presentation was responsible for the reduction in ^{51}Cr release. B3Z recognition of MEF-1 cells either expressing ovalbumin or pulsed with SIINFEKL peptide was blocked by γHV-68 infection (Fig. 2*A*); this difference from the earlier CTL assay (Fig. 1*C*) presumably reflected a higher threshold for hybridoma stimulation than for cell-mediated cytotoxicity. Antigen presentation from a retrovirus-expressed M-TSINFVKI-ASNENMETM-SIINFEKL polytope (MEF-P) was also inhibited, although not completely so, by γHV-68 infection (Fig. 2*B*). Mixing infected and uninfected targets (Fig. 2*B*) established that the inhibition of antigen presentation was limited to γHV-68-infected cells. Thus the simple presence of infected cells did not compromise B3Z function, and the involvement of a soluble factor such as a viral IL-10 homolog (5) was unlikely.

Infection with γHV-68 Leads to Diminished Cell-Surface MHC Class I Expression. The capacity of γHV-68 to block B3Z recognition of cells pulsed with exogenous peptide (Fig. 2*A*) suggested that fewer MHC class I molecules were present on virus-infected targets. This possibility was confirmed by flow cytometric analysis of γHV-68-infected MEF-1 cells, which showed down-regulation of both H-2D^b and H-2K^b (Fig. 3 *A* and *B*). The effect was not promoter-specific, inasmuch as there was also down-regulation of H-2K^b expressed from a pMSCV-H-2K^b-NEO retrovirus in BALB/c-3T3 cells (Fig. 3*C*) or BHK-21 cells (Fig. 3*E*). Native H-2K^d expression on BALB/c-3T3 cells was also reduced (Fig. 3*D*). Infection with γHV-68 infection is predominantly lytic in all of these cell lines.

Although γHV-68-infected fibroblasts appeared viable, excluded trypan blue, and retained ^{51}Cr (Fig. 1) for at least 24 h, infection had a profound effect on host protein synthesis (Fig. 4*A*), raising the possibility that the loss of surface MHC class I was a consequence of a virus-induced host shutoff. However, phosphonoacetic acid treatment to block viral DNA synthesis, and hence late gene expression, significantly inhibited host shutoff (Fig. 4*A*) without affecting MHC class I down-regulation (Fig. 4*B*). Thus the reduction in cell-surface MHC class I appeared to be a selective effect.

Identification of a Viral Gene That Blocks Antigen Presentation. The next step was to identify γHV-68 genes responsible for inhibiting antigen presentation. Viral genomic library clones in pUC119 (9) were transfected into L929-H-2K^b cells, together with the major immediate-early viral transactivator (ORF 50) (27) and an M-TSINFVKI-ASNENMETM-SIINFEKL polytope, each in pCDNA3. After 48 h, the presentation of H-2$K^{b\text{-SIINFEKL}}$ was assayed using B3Z. The m152 gene of murine cytomegalovirus, which retains newly formed MHC class I molecules in the endoplasmic reticulum (4), provided a positive control. The γHV-68 ORF 50 by itself caused only a minor reduction in SIINFEKL presentation in this transient transfection assay, which was most likely due to some cellular toxicity (data not shown). No γHV-68 genomic clone inhibited presentation in the absence of ORF 50, emphasizing that this gene has a crucial role in up-regulating γHV-68 gene expression.

The *Hin*dIII-I genomic fragment of γHV-68 (9) consistently reduced hybridoma stimulation by the cotransfected polytope

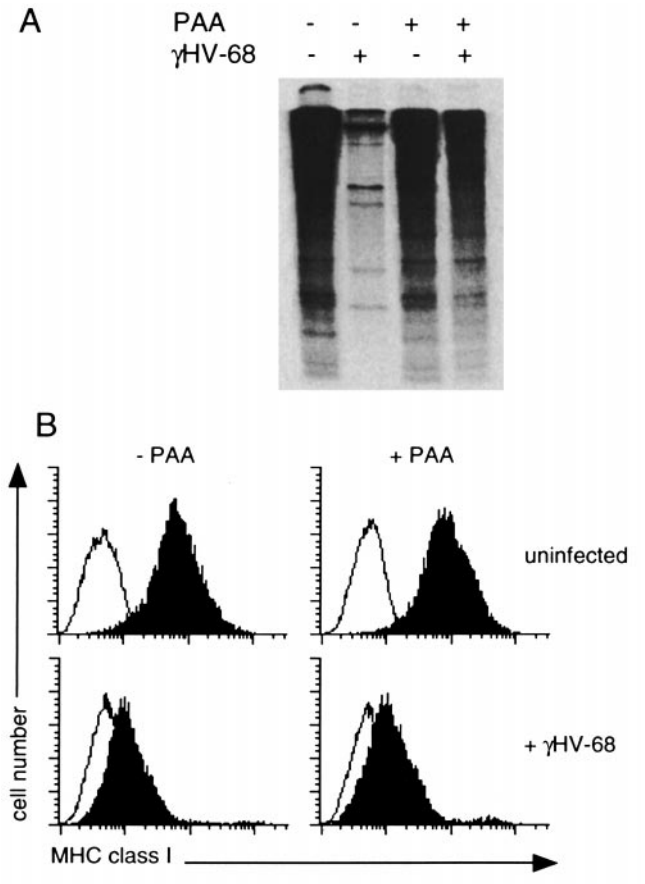

Fig. 4. The effect of phosphonoacetic acid treatment on viral host shutoff and MHC class I down-regulation. H-2^b MEFs were either left uninfected or infected overnight with MHV-68 (10 pfu per cell) and exposed or not to 100 μg/ml phosphonoacetic acid (PAA), starting 1 h before infection. (*A*) Cells were assayed for total cellular protein synthesis by [^{35}S]methionine labeling, followed by SDS/PAGE of total protein extracts. (*B*) Cells from parallel cultures were stained for surface MHC class I expression, using anti-H-2K^b (filled curves), or were stained with secondary antibody alone (open curves). Equivalent results of the H-2K^b staining were obtained by using anti-H-2D^b.

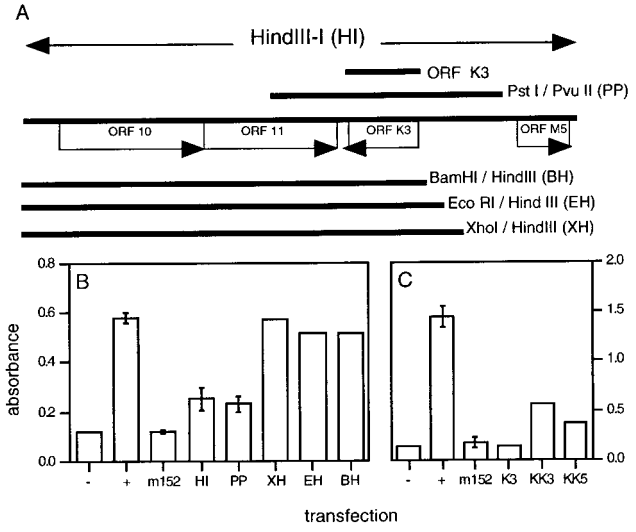

Fig. 5. Identification of a γHV-68 gene that blocks antigen presentation. (*A*) The *Hin*dIII-I fragment of γHV-68 is shown, together with the viral ORFs it contains (10) and the extents of the other viral fragments used for transfection. In each case, H-2K$^{b\text{-SIINFEKL}}$ antigen presentation was assayed, using β-galactosidase production by the B3Z hybridoma. (*B*) L929-H-2K^b cells were transfected with pCDNA3-SIINFEKL polytope (1 μg), pCDNA3-ORF 50 (1 μg), and a genomic fragment as indicated (1 μg), either in pUC-119 or in pSV40-ZEO (Invitrogen). HI, PP, XH, EH, and BH refer to the γHV-68 genomic DNA fragments indicated in *A*. For "no epitope," cells were transfected with pCDNA3-ORF 50 (1 μg) plus empty pCDNA3 vector (2 μg). For "no inhibitor," cells were transfected with pCDNA3-polytope (1 μg), pCDNA3-ORF 50 (1 μg), and empty pCDNA3 vector (1 μg). As a positive control, cells were transfected with pCDNA3-polytope (1 μg), pCDNA3-ORF 50 (1 μg), and pCDNA3 containing the m152 inhibitory gene of murine cytomegalovirus (1 μg). (*C*) L929-H-2K^b cells were transfected with pCDNA3-polytope (1 μg) plus the K3 genomic ORF (K3) or the homologous KSHV K3 (KK3) or K5 (KK5) ORFs, each expressed in PCDNA3 (1 μg). Because all expression was driven by the cytomegalovirus immediate-early promoter in pCDNA3 for this experiment, pCDNA3-ORF 50 was not included in the transfection.

(Fig. 5 *A* and *B*). *Hin*dIII-I contains at least four viral genes (ORF 10, ORF 11, ORF K3, and ORF M5), but a *Pvu*II–*Pst*I subfragment that contains only the K3 ORF was also fully effective (Fig. 5*B*). Further truncations of the 5′ region upstream of the K3 ORF curtailed the inhibition. Thus, the ORF 50-responsive K3 promoter probably has crucial elements between the upstream *Pvu*II and *Xho*I restriction sites. Cloned ORF K3 in pCDNA3 reproduced the inhibition of antigen presentation (Fig. 5*C*), indicating that this contains the entire essential coding sequence. The K3 ORF is relatively conserved among γ2-herpesviruses, and the homologous K3 and K5 ORFs of KSHV also reduced the presentation of H-2K$^{b\text{-SIINFEKL}}$ (Fig. 5*C*).

Analysis of stable, retrovirus-transduced cell lines indicated that γHV-68 K3 decreased H-2 class I antigen expression, whereas KSHV K3 and KSHV K5 down-regulated HLA class I (Fig. 6 *C*, *F*, and *J*). KSHV K3 also reduced H-2 class I expression somewhat (Fig. 6 *E* and *G*), whereas, surprisingly, given its effectiveness in the transient transfection assay, KSHV K5 had little such effect (Fig. 6 *I* and *K*). γHV-68 K3 down-regulated both HLA and H-2 in murine L-929 cells but affected neither in human 293 cells (Fig. 6 *A–D*). Pulse–chase metabolic labeling of H-2D^b glycoproteins synthesized in murine RMA cells transduced with pMSCV-γHV-68 K3-NEO (Fig. 7) indicated that, in the presence of K3, the half-life of the H-2D^b molecule is reduced to less than 15 min. No progression of H-2D^b to an endoglycosidase H-resistant form was observed.

Discussion

The present analysis establishes that γHV-68, a γ2-herpesvirus, blocks MHC class I-restricted antigen presentation and evades recognition by antigen-specific CTLs. The viral K3 ORF alone was sufficient to reduce cell-surface MHC class I expression and to inhibit antigen presentation. K3 may not be the only γHV-68 gene involved, but screening assays to date have not identified another γHV-68 genomic fragment with an efficacy comparable to that of *Hin*dIII-I, which contains K3. A K3-deficient virus is currently being generated to determine whether this gene is necessary for CTL evasion and to establish whether K3 expression is indeed responsible for the failure of CD4$^+$ T cell-deficient mice to control γHV-68.

A K3 homolog is present in the same genomic location of several γ2-herpesviruses, for example, the major immediate-early gene of bovine herpesvirus-4 (28), ORF 12 of *Herpesvirus saimiri*, and the K3 and K5 genes of KSHV (29). The fact that both KSHV homologs also down-regulated antigen presentation suggests a conserved family of immune evasion genes. Each K3 homolog includes a PHD/LAP zinc finger (29), a motif that also appears in a variety of cellular proteins (30); the closely related zinc-binding RING finger is involved in ubiquitin ligase recruitment (31), but whether this is also true of PHD/LAP fingers is unknown. Inasmuch as γHV-68 K3 inhibited HLA-A2 expression in murine cells but not murine H-2K^b expression in human

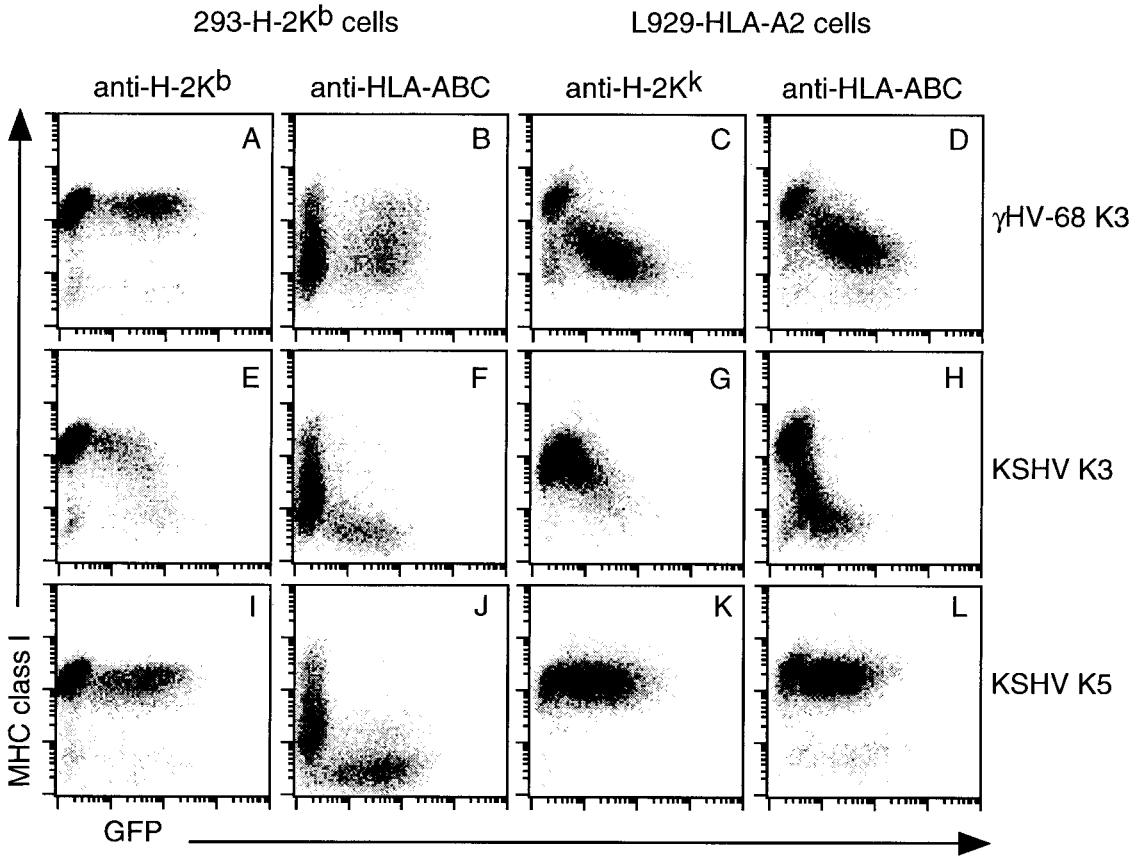

Fig. 6. Down-regulation of surface MHC class I expression by γHV-68 K3 and its KSHV homologs. The γHV-68 K3, the KSHV K3, and the KSHV K5 ORFs were cloned into pMSCV-GFP and expressed by retroviral transduction, as indicated in human 293 cells stably transfected with murine H-2K^b and murine L929 cells stably transfected with human HLA-A2. GFP$^+$ transduced cells were then mixed with GFP$^-$ untransduced cells to compare MHC class I expression, in each case using a phycoerythrin-conjugated goat anti-mouse IgG secondary antibody.

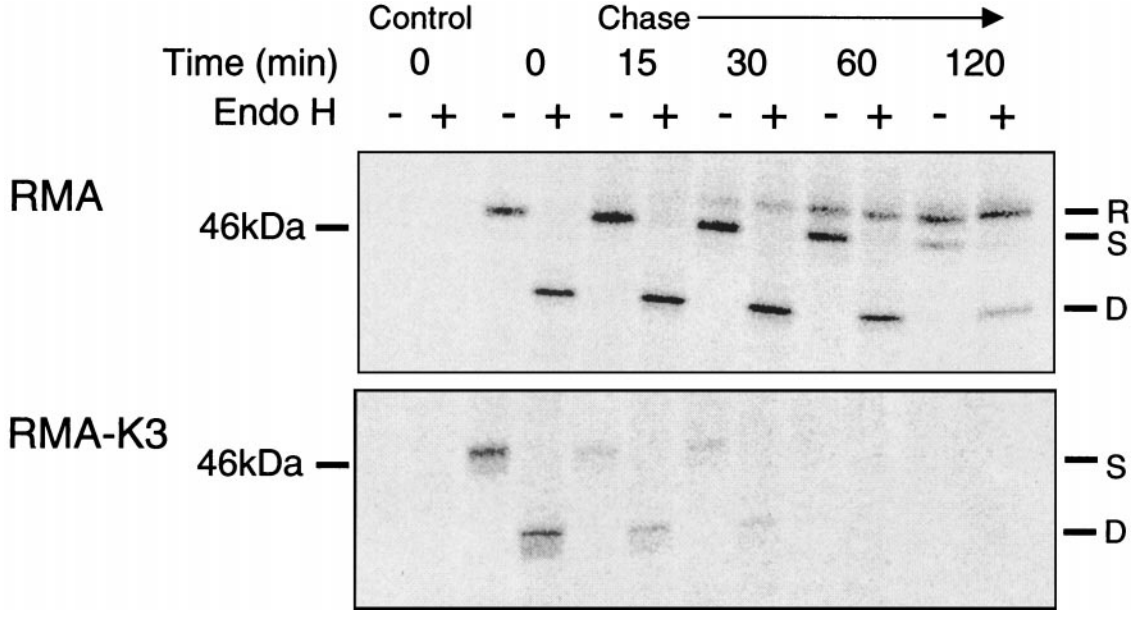

Fig. 7. Short half-life of MHC class I in cells expressing the γHV-68 K3. Murine RMA cells and RMA cells transduced with pMSCV-γHV-68 K3-NEO retrovirus (RMA-K3) were pulse-labeled for 15 min with [^{35}S]methionine and then chased for the time indicated. H-2D^b molecules were immunoprecipitated with 28.14.8 antibody plus protein A-Sepharose, then digested (+) or not (−) with endoglycosidase H. Control samples were pulse-labeled for 15 min and immunoprecipitated at time point 0 without specific antibody. The bands observed correspond to endoglycosidase H-resistant (R), sensitive (S), and digested (D) H-2D^b.

cells (Fig. 6), it must have a necessary and species-specific interaction with a non-MHC class I protein. This interaction has yet to be identified.

The pulse–chase analysis of H-2D^b in K3-transduced cells showed a rapid loss of 28.14.8-reactive molecules with no progression to an endoglycosidase H-resistant form (Fig. 7). These results suggested that γHV-68 K3 causes the destruction of newly synthesized MHC class I proteins. The very rapid disappearance of H-2D^b in the presence of γHV-68 K3 was reminiscent of the effects of the US2 and US11 proteins of cytomegalovirus, both of which export HLA antigens from the endoplasmic reticulum to be degraded in the cytosol (32, 33). Interestingly, the KSHV K5 protein is known to be expressed in the endoplasmic reticulum (34), where MHC class I/β_2-microglobulin/peptide complexes are first assembled. Precisely how γHV-68 K3 and its homologs work has not yet been determined.

How can we reconcile a strong *in vivo* CTL response to γHV-68 with an inhibition of antigen presentation? Substantial populations of CD8$^+$ T cells recognizing lytic cycle epitopes can be recovered from γHV-68-infected mice, even in the absence of CD4$^+$ T cell help (17). However, these effectors achieve only partial control of productive viral replication (16). Presumably some infected cells do present lytic cycle antigens to CTL *in vivo*: the viral inhibitory mechanisms may be overcome by cytokine stimulation or may be ineffective in certain cell types. The actual generation of CTLs may depend to a large degree on cross-priming (35). In broad terms, the γ2-herpesviruses probably employ a strategy similar to that of α- and β-herpesviruses of reducing antigen presentation during lytic cycle infection, such that CTLs cannot comprehensively prevent new virion production. The potential to use murine γHV-68 to relate *in vitro* gene function to *in vivo* pathogenesis underlines the usefulness of this experimental system for understanding the crucial events in γ-herpesvirus immunity.

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