An Early Event in Murine Cytomegalovirus Replication Inhibits Presentation of Cellular Antigens to Cytotoxic T Lymphocytes

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Cytomegalovirus (CMV) infection of simian virus 40 (SV40)-immune mice inhibits priming of SV40-specific helper and cytotoxic T lymphocytes (CTL) in vivo (A. E. Campbell, J. S. Slater, and W. S. Futch, Virology 173:268-275, 1989; J. S. Slater, W. S. Futch, V. J. Cavanaugh and A. E. Campbell, Virology 185:132-139, 1991). We now demonstrate that murine CMV (MCMV) infection of SV40-transformed macrophages and fibroblasts prevents presentation of SV40 T antigen to SV40-specific CTL. MCMV-infected macrophages failed to stimulate SV40-immune CTL precursors in vitro. In addition, MCMV-infected, SV40-transformed macrophage and fibroblast target cells lost their susceptibility to lysis by major histocompatibility complex class I-restricted, SV40-specific CTL clones. MCMV infection did not alter the synthesis of SV40 T antigen in the target cells. MCMV early gene expression was required for inhibition of SV40 T-antigen presentation; immediate-early gene expression was insufficient for this effect. Early viral gene expression also resulted in a significant reduction of H-2K and H-2D molecules on the surface of MCMV-infected fibroblasts. However, this reduction occurred independently from suppression of antigen presentation to CTL. The same target cells which were resistant to lysis by SV40 CTL were susceptible to lysis by MCMV-specific CTL. MCMV early gene products therefore interfere with the processing and/or presentation of SV40 T-antigen determinants to CTL independent of alterations in the major histocompatibility complex.

Our previous studies aimed to identify mechanisms of cytomegalovirus (CMV)-induced suppression of cell-mediated immunity. We demonstrated that acute infection of simian virus 40 (SV40)-immune mice prevents priming of SV40-specific cytotoxic T lymphocyte (CTL) precursors (6). This occurs when murine CMV (MCMV) infection either precedes or follows SV40 immunization. Continued studies by Slater et al. revealed that priming of T-helper lymphocytes in response to SV40 immunization is also disrupted by MCMV infection (24). Lack of T-helper cell function in MCMV-infected mice does not, however, explain the deficiency in CTL priming, as this latter event is T-helper cell independent (24). Therefore, MCMV infection must inhibit an event common to cytotoxic and helper T-lymphocyte priming, such as antigen presentation.

Elegant studies by Del Val et al. (7) describe a selective inhibition in presentation of MCMV immediate-early (IE) protein, pp89, to H-2Ld-restricted, pp89-specific CTL clones. An MCMV early (E) gene product is responsible for this effect. E gene expression does not affect the synthesis or stability of pp89, and there is no concomitant alteration in expression of major histocompatibility complex (MHC) class I molecules. This was the first indication that an MCMV protein(s) could interfere with the process of antigen presentation. We questioned whether such inhibition in antigen presentation by MCMV is selective for MCMV peptides or whether presentation of other cellular peptides is also affected by the virus. This is an important question, because in vivo, MCMV infects macrophages and other antigen-presenting cells (3, 20, 22).

We addressed the question of whether MCMV interferes with presentation of normal host cell proteins by assessing the effects of MCMV infection of SV40-transformed cells on the ability of these cells to present SV40 T antigen to SV40-specific, MHC class I-restricted CTL. SV40 T antigen is constitutively expressed in the transformed cells and thereby mimics a host cell protein. The T antigen is processed into at least five distinct peptide determinants which are recognized by SV40 CTL (see reference 28 for a review).

We tested the effects of MCMV infection of SV40-transformed macrophages and fibroblasts on presentation of SV40 T antigen to SV40-specific CTL and determinant-specific CTL clones. The data indicate that an MCMV E gene product prevents presentation of T antigen to CTL. This inhibition does not rely on a reduction in surface MHC class I molecules, although an MCMV E gene product(s) does reduce surface MHC expression. Furthermore, MCMV infection has no effect on the synthesis of SV40 T antigen in the transformed cells. MCMV proteins expressed in infected, SV40-transformed cells are presented to MCMV-specific CTL when SV40 T antigen is not presented. Therefore, MCMV infection interferes with processing and/or presentation of cellular proteins by some mechanism independent of reduced surface MHC expression.

MATERIALS AND METHODS

Cells. SV40-transformed H-2b fibroblasts (designated WT-19) and SV40-transformed H-2d fibroblasts (mKSA) were obtained from Satvir S. Tevethia (Pennsylvania State University College of Medicine) and maintained as previously described (6). SV40-transformed H-2d-macrophages (IC-21) were obtained from the American Type Culture Collection (Rockville, Md.) and were maintained as previously described (19). SV40-specific H-2d-restricted CTL clones K11 and K19 have been previously described (5).

Virus. Preparation of salivary gland-derived MCMV and tissue culture-passaged stocks has been described (6). All cells were infected with a multiplicity of 1 to 2 PFU per cell for 24 h unless stated otherwise. Mock infections employed
either salivary gland homogenates from noninfected mice or cell culture supernatants from noninfected NIH 3T3 cells.

**Drug-restricted replication of MCMV.** The use of phos- phonoacetilic acid (PAA), cycloheximide (CH), and actino- mycin D (ActD; Sigma Chemical Co., St. Louis, Mo.) to selectively restrict MCMV replication has previously been described (7). Briefly, cells were treated with 250 μg of PAA per ml at the time of virus infection for a duration of 24 h. Cells were treated with 50 μg of CH per ml at the time of infection for a total of 3 h. CH was subsequently washed from the cells, and incubation continued for another 4 h either in media alone (CH−) or in media containing 100 μg of ActD per ml (CH/ActD).

**CTLp frequency analysis.** Detailed procedures for SV40 CTL precursor (CTLp) frequency analysis have been described previously (6). Briefly, limiting numbers of SV40-immune lymph node cells (LNC) were cultured for 7 days with mitomycin-treated WT-19 stimulator cells, irradiated splenocytes as filler cells, recombinant interleukin-2 (rIL-2), and other growth factors.

**Cytotoxicity assays.** 31Cr-release cytotoxicity assays were performed as previously described (26). All target cells were treated with 20 U of gamma interferon (kindly supplied by The American Cancer Society, New York, N.Y.) per ml 24 h prior to MCMV infection and during the 24-h infection. This dose of interferon does not inhibit MCMV replication in C57BL/6 fibroblasts (18).

**Quantitation of surface MHC class I expression.** Surface expression of MHC class I molecules was quantitated on WT-19 fibroblasts by flow cytometry as previously described (24). Percent positive cells (5,000 total) was calculated on the basis of forward angle light scatter versus log-inte- grated 90° light scatter by using the Immuno Program, Coulter MDADS I (Hialeah, Fla.). Primary antibodies were as follows: 28-14-8S (mouse immunoglobulin G2a [IgG2a] anti-H-2Dk, provided by Richard Ciavarra of our department), Y-3 (mouse IgG2b anti-H-2Kk, provided by James Shell, West Virginia University), and Ly-ml (mouse IgG2b anti-β-m, provided by Ulrich Hammerling, Sloan-Kettering Institute). All antibodies were used at approximately 1 μg/10⁶ cells. Isotype-matched mouse IgG served as a control. Fluorescein isothiocyanate-conjugated goat anti-mouse IgG and IgM (Boehringer Mannheim, Indianapolis, Ind.) served as the secondary antibody.

**MCMV-specific CTL.** Polyclonal MCMV CTL were generated from LNC harvested from mice 9 to 10 days after infection with 10⁵ PFU of MCMV via the footpad. LNC (7 × 10⁶ cells per microtiter well) were incubated with 2 × 10⁵ stimulator cells, 3 × 10⁵ gamma-irradiated spleen cells, 1 U of rIL-2 (AmGen Inc., Thousand Oaks, Calif.), and 5% rat T-cell Polyclone (Collaborative Research, Bedford, Mass.). Stimulator cells were WT-19 cells infected for 24 h with MCMV and then treated with 25 μg of mitomycin (Sigma) per ml.

**Synthesis of SV40 T antigen.** To determine if MCMV gene products altered the synthesis of SV40 T antigen, lysates of infected WT-19 cells were analyzed by immunoprecipitation, using modifications of the technique described by Finlay et al. (8). WT-19 cells were seeded at 5 × 10⁵ cells per 100-mm plate and 24 h later were mock infected or infected with MCMV at a multiplicity of 3 to 4 PFU per cell. At various times after infection, cells were starved in methionine-free medium for 1 h, and labeled for 2 h with 100 μCi of [35S]methionine (DuPont NEN Research Products, Reston, Va.) per ml in methionine-free medium containing 2% fetal bovine serum. At 3, 8, and 24 h after infection, cell lysates were prepared by washing cells with Tris-buffered saline (30 mM Tris-hydrochloride [pH 7.4], 150 mM NaCl), followed by lysis in 1.0 ml of immunoprecipitation lysis buffer (14) for 30 min. Cell lysates were clarified by centrifugation and precleared with mouse IgG (Cappel Organon Teknika Corp., Durham, N.C.) and 50% protein A-Sepharose (Sigma) overnight at 4°C. For immunoprecipitations, control mouse IgG and antibody PAb901 (kindly provided by Satvir S. Te- vethia), which recognizes the carboxy terminus of SV40 T antigen (27), were used. Proteins were analyzed by employing two approaches for immunoprecipitation: (i) use of cell lysates containing equal trichloroacetic acid-precipitable counts (12 × 10⁶ cpm) or (ii) use of equal volumes of cell lysates. With respect to the latter approach, this represents an approximately equal number of infected cells at each time after infection. Because the mock-infected cells were harvested at 24 h, there were approximately two times the number of cells compared with the infected cell samples. Similar results were obtained with either method. Immune complexes were washed once with SNNTF buffer (5% sucrose, 1% Nonidet P-40, 0.5 M NaCl, 50 mM Tris-HCl [pH 7.4], 5 mM EDTA) and three times with radiolymphoprecipitation assay buffer (14) and were separated on a 10% polyacrylamide gel as previously described (25).

**Steady-state levels of SV40 T antigen.** Lysates from equal numbers of mock-infected and MCMV-infected WT-19 cells were analyzed with antibody PAb901 by Western immuno- blot as previously described (21). Primary antibody binding was detected by using alkaline phosphatase-conjugated goat anti-mouse antibody (Promega Biotech, Madison, Wis.) and bands were visualized by chemiluminescence according to manufacturer’s instructions (Tropix, Bedford, Mass.).

**RESULTS**

Effects of MCMV infection on presentation of SV40 T antigen to SV40 CTL. Our earlier studies (6, 24) indicated that MCMV infection of mice prevented priming of both T-helper lymphocytes and CTL in vivo. We therefore inves- tigated the effects of MCMV infection on presentation of SV40 T antigen to CTL by two methods. First, the ability of MCMV-infected, SV40-transformed cells to stimulate SV40 CTL precursors was analyzed. This approach relies on presentation of SV40 T antigen to SV40-immune CTL precursors primed in vivo. Presentation is then assessed by quantitating the frequency of primed CTLp which proliferate to mature, effector CTL in response to antigen and growth factors. The results in Table 1 indicate that MCMV-infected IC-21 macrophages were poor stimulators of SV40 CTL compared with mock-infected cells. The frequency of SV40 CTLp generated in response to MCMV-infected stimulator cells was comparable to the frequency generated in the absence of antigen. The suppression was not due to an inhibitory substance produced by infected IC-21 macrophages, since CTLp cultured with both mock-infected and MCMV-infected stimulator cells yielded a frequency as high as those cultured with mock-infected stimulator cells alone. These results indicated that MCMV infection interfered with the ability of SV40-transformed stimulator cells to present SV40 T antigen to CTL. Additional experiments revealed that in vitro stimulation of SV40 T-antigen-dependent CTL is MHC class I restricted. When syngeneic (H-2b) and allo- geneic (H-2d) SV40-transformed stimulator cells were com- pared in their abilities to stimulate SV40 CTL, the allogeneic cells were significantly less efficient as stimulators (data not shown).
TABLE 1. Frequency of SV40-specific CTL generated in response to MCMV-infected stimulator cells

<table>
<thead>
<tr>
<th>Stimulator cellsb</th>
<th>Reciprocal frequency (95% confidence range)(^c) with following target cells:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT-19 (SV40, H-2(^b))</td>
</tr>
<tr>
<td>MOCK IC-21</td>
<td>348 (263–514)</td>
</tr>
<tr>
<td>MCMV IC21</td>
<td>5,115 (2,936–19,831)</td>
</tr>
<tr>
<td>MOCK + MCMV IC21</td>
<td>206 (153–318)</td>
</tr>
<tr>
<td>None</td>
<td>2,047 (1,530–3,092)</td>
</tr>
</tbody>
</table>

\(\ast\) Popliteal LNC were harvested from C57BL/6 (H-2\(^b\)) mice 10 to 11 days after immunization via the footpad with SV40 (10\(^5\) PFU). LNC were cultured in limiting dilution with stimulator cells (2 \times 10\(^5\) per well), irradiated spleen cells (3 \times 10\(^5\) per well), rIL-2 (1 U per well), and 10% supernatant from concanavalin A-stimulated rat spleen cells.

b LNC were cultured with mock-infected IC-21 macrophages (MOCK IC-21), IC-21 cells infected for 24 h with 1 PFU of MCMV per cell (MCMV IC21), equal numbers of mock- and MCMV-infected IC-21 cells (MOCK + MCMV IC21), or no stimulator cells (None).

c After 7 days of incubation, cytotoxicity activity was assessed by a \(^{51}\)Cr-release assay with the indicated target cells. Wells scored as positive for cytotoxicity had counts per minute greater than 3 standard deviations from the mean of control wells cultured without effector LNC. Minimal chi-square analysis was used to obtain CTL frequencies on the basis of the number of negative wells per effector LNC concentration.

Results from a second series of experiments further indicated that MCMV infection inhibits MHC class I-restricted presentation of SV40 T antigen to SV40 CTL. MCMV-infected, SV40-transformed IC-21 macrophages and WT-19 fibroblasts were tested for their susceptibilities to lysis by SV40-specific, H-2\(^d\)-restricted CTL clones K11 and K19. These CTL clones recognize two distinct determinants on the amino terminus of SV40 T antigen, sites I and II, respectively (1, 28). The data in Fig. 1 illustrate the relative susceptibilities of MCMV-infected and mock-infected target cells to lysis by the SV40 CTL clones. The results indicate that MCMV infection of SV40-transformed macrophages and fibroblasts abolishes their susceptibilities to lysis by both antigen-specific CTL.

Identification of MCMV gene products responsible for inhibition of antigen presentation. The relative roles of MCMV IE, E, and late (L) gene products in interfering with antigen presentation were subsequently examined. The above experiments were repeated by using SV40-transformed target cells infected with MCMV in the presence of various inhibitors of gene expression to selectively restrict virus replication. The drug PAA prevents L gene expression by inhibiting the viral DNA polymerase and consequently restricts replication to the IE and E phases of replication. Use of CH followed by ActD (CH/ActD) restricts replication to the IE phase, whereas CH treatment and its subsequent removal, without further drug treatment (CH/–), allows expression of E proteins. The results in Fig. 2b indicate that newly synthesized L gene products are not required for inhibition of antigen presentation. PAA treatment had no effect on the ability of MCMV to interfere with recognition by SV40 CTL.

![FIG. 1. Effects of MCMV infection on target cell susceptibility to SV40-specific CTL. SV40-transformed fibroblasts (WT-19) and macrophages (IC-21) were mock infected or infected with 1 PFU of MCMV per cell for 24 h and labeled with \(^{31}\)Cr. Target cells were incubated with SV40 CTL clones K11 and K19 at the indicated effector-to-target cell ratios for the indicated times (5 or 18 h). Cytotoxicity is expressed as percent specific release.](image-url)

![FIG. 2. Effects of restricted MCMV replication in SV40-transformed target cells on susceptibility to lysis by SV40-specific CTL. SV40-transformed fibroblasts (WT-19) (\(\bullet\), \(\bigcirc\)) and macrophages (IC-21) (\(\bullet\), \(\square\)) were mock infected (closed symbols) or infected with 1 PFU of MCMV per cell (open symbols). The target cells were either untreated or treated with PAA for 24 h, CH for 3 h, or CH for 3 h and then with ActD for 4 h. Target cells were subsequently labeled with \(^{31}\)Cr and incubated with SV40-specific CTL clone K19 at the indicated effector-to-target cell ratio for 5 h.](image-url)
These results also eliminate the direct role for input virus infection of MCMV-infected cells used in the legend to Fig. 2. Because MCMV-specific CTL. SV40-transformed target cells were infected and drug treated as described in the legend to Fig. 2. Polyclonal MCMV CTL were generated from LNC harvested from mice 9 to 10 days after infection with 10³ PFU of MCMV via the footpad. The LNC were cultured with mitomycin-treated, MCMV-infected WT-19 cells and 5 U of rIL-2 per ml for 7 days. Target and effector cells were incubated at the indicated ratios for 5 h. Symbols are as described in the legend to Fig. 2.

The use of CH/ActD and CH/- distinguished the relative roles of MCMV IE and E proteins in regulating antigen presentation. MCMV E proteins were clearly involved in suppression of antigen presentation, since suppression was significant when target cells were treated with CH without subsequent ActD treatment (Fig. 2c). Suppression was minimal when replication was restricted to the IE phase with CH/ActD treatment (Fig. 2d). It is important to note that CH- and CH/ActD-treated cells were infected with MCMV for a total of only 7 h, compared with the 24 h infection of PAA-treated cells. This explains the differences in the magnitude of suppression of lysis by MCMV infection in the presence of PAA versus CH/- and CH/ActD. The half-life of peptide-MHC complexes on the cell surface is approximately 14 h. Therefore, preexisting SV40 T-peptide-MHC complexes are contributing to the lysis of target cells infected with MCMV for 7 but not 24 h. Collectively, the above data indicate that MCMV E proteins are required for maximal inhibition of class I-mediated antigen presentation. These results also eliminate at least a direct role for input virion proteins in inhibiting antigen presentation.

**Presentation of MCMV antigens to MCMV-specific CTL.** Because MCMV E proteins appeared to be involved in regulating expression of surface SV40 T-antigen-MHC complexes, we tested the infected target cells for presentation of MCMV antigens. The same population of SV40-transformed, MCMV-infected cells used in experiments in Fig. 2 was also used as targets of MCMV-immune CTL. The results in Fig. 3 indicate that MCMV antigens were indeed presented on the surface of cells proven to be devoid of SV40 T-antigen recognition. Again, MCMV E proteins, as opposed to IE or L viral proteins, were the major determinants recognized by H-2b MCMV-specific CTL. MCMV-infected target cells treated with PAA (Fig. 3b) or CH/- (Fig. 3c) were competent targets, whereas CH/ActD-treated cells (Fig. 3d) were poor targets for MCMV CTL. These observations confirm those of Del Val et al., who demonstrated that H-2b C57BL/6 cells selectively expressing MCMV IE proteins are relatively poor targets for polyclonal anti-MCMV CTL (7).

**Effects of MCMV infection on MHC class I expression.** We next addressed the possibility that MHC class I molecules were limited in MCMV-infected cells. This was an unlikely explanation, however, for the following reasons. (i) MCMV antigens were efficiently presented to MCMV CTL; therefore, sufficient MHC molecules were present on infected targets. (ii) The number of MHC class I (4) and class II (13) molecules needed for antigen presentation is quite small; therefore, a reduction in surface MHC molecules would not necessarily result in a functional decrease in CTL lysis. (iii) The MCMV-infected target cells were treated with gamma interferon to maintain maximal expression of MHC molecules.

However, it remained possible that MCMV infection suppressed MHC class I expression and that limited MHC molecules favored competition between MCMV and SV40 T-antigen peptides. MHC class I expression was quantitated on the surface of MCMV infected, WT-19 fibroblasts treated with gamma interferon for various time periods. The data in Table 2 indicate that MCMV infection suppresses MHC surface expression unless gamma interferon treatment continues throughout infection. Also, the degree of suppression depended on the quantity of MHC expressed prior to infection. For example, MHC expression on cells treated with interferon for 48 h prior to infection was less affected by virus infection compared with that of cells treated with interferon for only 24 h and expressing suboptimal levels of MHC class I. Most importantly, the results indicate that inhibition of SV40 T-antigen presentation on MCMV-infected cells occurs independently of alterations in MHC class I expression. MCMV-infected target cells used in all cytotoxicity experiments were treated with gamma interferon 24 h prior to and 24 h during MCMV infection. As shown in Table 2, this treatment does not result in significant reduction in surface MHC heavy or light chain molecules.

**Table 2. Effects of gamma interferon treatment on MCMV-induced suppression of MHC class I expression.**

<table>
<thead>
<tr>
<th>Surface molecule</th>
<th>Ext</th>
<th>24 h prior</th>
<th>48 h prior</th>
<th>24 h prior and after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MOCK</td>
<td>MCMV</td>
<td>MOCK</td>
<td>MCMV</td>
</tr>
<tr>
<td>H-2D*</td>
<td>1</td>
<td>79</td>
<td>20</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>89</td>
<td>34</td>
<td>94</td>
</tr>
<tr>
<td>H-2K*</td>
<td>1</td>
<td>77</td>
<td>20</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>89</td>
<td>49</td>
<td>98</td>
</tr>
<tr>
<td>B2m</td>
<td>2</td>
<td>79</td>
<td>40</td>
<td>91</td>
</tr>
</tbody>
</table>

* Surface expression of MHC class I molecules was quantitated by immunofluorescence with the following primary antibodies: H-2D*, 2B-14-85; H-2K*, Y-3; B2m, Ly-m11 and fluorescein isothiocyanate-labeled goat anti-mouse IgG and IgM.

**FIG. 3.** Effects of restricted MCMV replication in SV40-transformed target cells on susceptibility to lysis by MCMV-specific CTL. SV40-transformed target cells were infected and drug treated as described in the legend to Fig. 2. Polyclonal MCMV CTL were generated from LNC harvested from mice 9 to 10 days after infection with 10³ PFU of MCMV via the footpad. The LNC were cultured with mitomycin-treated, MCMV-infected WT-19 cells and 5 U of rIL-2 per ml for 7 days. Target and effector cells were incubated at the indicated ratios for 5 h. Symbols are as described in the legend to Fig. 2.
We further questioned the relative roles of MCMV IE, E, or L gene products in downregulating surface MHC expression. WT-19 cells were pretreated for 24 h with gamma interferon and then infected with MCMV in the presence of the various drugs described above. The results in Table 3 indicate that MCMV E protein expression was required for maximal suppression of MHC expression. However, results with CH/- and CH/ActD treatments are difficult to interpret, because these drugs affected MHC expression on mock-infected cells and the percentage of cells expressing the MHC molecules was low.

**Effects of MCMV infection on expression of SV40 T antigen.**

One possible explanation for a reduction in presentation of SV40 T antigen, independent of MHC class I expression, is downregulation of SV40 T-antigen expression by MCMV gene products. Processed peptides recognized by SV40 CTL are likely derived from newly synthesized SV40 T antigen. Therefore, we compared the synthesis of T antigen in WT-19 cells at various stages of MCMV infection. Radiolabeled cell lysates were prepared at 3 (IE), 8 (E), and 24 (L) h after MCMV infection (7) and were analyzed by immunoprecipitation with a monoclonal antibody (PAb901) specific for the carboxy terminus of SV40 T antigen.

The results in Fig. 4 represent immunoprecipitations of lysates, based on trichloroacetic acid-precipitable counts. MCMV infection at IE, E, and L times did not decrease the synthesis of SV40 T antigen relative to total radiolabeled cell protein. When the experiment was repeated with equal volumes of cell lysates, again no MCMV-induced reduction in the expression of T antigen was observed (data not shown). In fact, the data in Fig. 4 suggest that synthesis of T antigen increases early to late in infection. This is consistent with the studies of Pari and St. Jeor (21), who demonstrated that the major IE protein of human CMV (HCMV) causes an increase in the expression of SV40 T antigen.

We also compared the steady-state levels of SV40 T antigen in mock-infected and MCMV-infected WT-19 cells by Western blot analysis. The results in Fig. 5 indicate that MCMV infection for 24 h did not appreciably alter the steady-state level of this 92- to 95-kDa protein. The limitation of this method is that subtle changes in the stability of T antigen would not be detected by this method, because the half-life of T antigen in these transformed cells is approximately 22 h (28a). However, it was previously shown that even a major reduction in the stability of SV40 T antigen (to 40 min) has no effect on CTL recognition (29).

**DISCUSSION**

The results from this study support our initial hypothesis, based on in vivo data, that MCMV infection interferes with antigen presentation. The findings of the present in vitro studies are likely applicable to the in vivo situation in which MCMV infection of mice prevents priming of helper T lymphocytes (24) and CTL (6). The results demonstrate that MCMV infection interferes with presentation of endogenously synthesized peptides. Antigen-presenting cells, such as macrophages and stromal cells of lymphoid organs, are permissive for productive and latent MCMV infection in vivo (3, 20, 22). MCMV infection could interfere with presentation of normal cellular peptides and with peptides from other infectious agents conointecting these cells. This is an important consideration for HCMV as well, because HCMV productively infects macrophages (15) and suppresses antigen-specific, MHC-restricted CTL activity (23).

Virus-induced perturbation in antigen-presenting functions has been reported for influenza virus (8), adenoviruses (for a review, see reference 11), vaccinia virus (4, 30), herpes

### TABLE 3. Effects of drug-restricted replication of MCMV on virus-induced suppression of MHC class I expression

<table>
<thead>
<tr>
<th>Surface molecule</th>
<th>% Positive target cells after following drug treatmenta</th>
<th>None</th>
<th>PAA</th>
<th>CH/-</th>
<th>CH/ActD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MOCK</td>
<td>MCMV</td>
<td>MOCK</td>
<td>MCMV</td>
</tr>
<tr>
<td>H-2Db</td>
<td></td>
<td>89</td>
<td>34</td>
<td>98</td>
<td>42</td>
</tr>
<tr>
<td>H-2Kc</td>
<td></td>
<td>89</td>
<td>49</td>
<td>97</td>
<td>56</td>
</tr>
<tr>
<td>β2m</td>
<td></td>
<td>79</td>
<td>40</td>
<td>92</td>
<td>43</td>
</tr>
</tbody>
</table>

a Surface immunofluorescence was determined by using the following primary antibodies: H-2Db, 28-14-8S; H-2Kc, Y-3; β2m, Ly-11 and fluorescein isothiocyanate-labeled goat anti-mouse IgG and IgM.

b Mock (MOCK) and MCMV-infected (MCMV) (1 FFU per cell) SV40-transformed fibroblasts were treated with PAA or control media (None) at the time of infection for 24 h. Other target cells were treated with CH (CH/-) or CH followed by ActD (CH/ActD) as described in Materials and Methods. Cells treated with CH and CH/ActD were infected for a total of 7 h.

FIG. 4. Effects of MCMV infection on the synthesis of SV40 T antigen. Equal numbers of SV40-transformed WT-19 fibroblasts were mock infected or infected with 3 to 4 FFU of MCMV per cell. At 3, 8, and 24 h after infection, lysates were prepared and analyzed by immunoprecipitation (as described in Materials and Methods) with an SV40 T-antigen-specific monoclonal antibody PAb901 (T) or control mouse IgG (C). Cell lysates were immunoprecipitated on the basis of trichloroacetic acid-precipitable counts of the mock-infected cell lysate prepared after 24 h. Protein sizes are indicated in kilodaltons.
viral virus product(s) disrupts antigen processing, i.e., peptide presentation, with protein gene class I or control and virus, Twenty-four hours after infection, equal numbers of cells were lysed, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and analyzed by Western blot as described in Materials and Methods. Primary antibody was monoclonal antibody PAb 901 or control mouse IgG. Antibody binding was visualized by using chemiluminescence.

FIG. 5. Effects of MCMV infection on steady-state levels of SV40 T antigen. SV40-transformed fibroblasts (WT-19) were mock infected (M) or infected with 1 PFU of MCMV per cell (CMV). Twenty-four hours after infection, equal numbers of cells were lysed, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and analyzed by Western blot as described in Materials and Methods. Primary antibody was monoclonal antibody PAb 901 or control mouse IgG. Antibody binding was visualized by using chemiluminescence.

class I molecules; however, such a reduction does not explain the lack of antigen presentation in MCMV-infected cells. Continual treatment with gamma interferon prevents MCMV-induced downregulation of MHC expression but does not overcome the interference with antigen presentation. The use of interferon therefore allows us to separate the MCMV-induced effects on MHC expression from the MCMV-induced suppression in antigen presentation. It is currently unknown whether MCMV infection affects the synthesis or transport of MHC molecules.

Because MCMV E gene products are involved in suppression of SV40 T-antigen presentation and at the same time are themselves presented to MCMV-specific CTLs, it is tempting to speculate that these viral gene products compete with SV40 T-antigen peptides for presentation by MHC class I molecules. Such a mechanism of competition between viral peptides for presentation at the cell surface has recently been proposed for vaccinia virus and lymphocytic choriomeningitis virus (4). However, it remains to be determined whether the same MCMV E proteins involved in suppression of antigen presentation are indeed the same viral proteins presented on the cell surface. The precise role of MCMV proteins in regulating antigen presentation is currently under investigation.

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