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The Murine Cytomegalovirus Immunomodulatory Gene m152 Prevents Recognition of Infected Cells by M45-Specific CTL But Does Not Alter the Immunodominance of the M45-Specific CD8 T Cell Response In Vivo

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Although in vitro studies have shown that herpesviruses, including murine CMV (MCMV), encode genes that interfere with the MHC class I pathway, their effects on the CTL response in vivo is unclear. We identified a D b -restricted CTL epitope from MCMV M45 by screening an MCMV genomic library using CTL clones isolated from mice infected with MCMV lacking m152. Because m152 severely inhibits CTL recognition of M45 in vitro, we questioned whether an M45-specific response would be generated in mice infected with wild-type MCMV expressing m152. Mice infected with wild-type MCMV or MCMVΔm152 made similar responses to the M45 Ag. Moreover, we saw no skewing of the proportion of M45-specific CD8 T cells within the total MCMV-infected mouse. Because pp89 is expressed before m4, m6, and m152, it is presumably less affected than E Ags are by these immunomodulatory genes. An epitope from the IE1 gene product pp89 is recognized by a substantial number of CD8 T cells in BALB/c mice (12–14). Because pp89 is expressed before m4, m6, and m152, it is presumably less affected than E Ags are by these immunomodulatory genes. The pp89 Ag is presented if gene expression is limited to IE genes; genes are classified as immediate early (IE), early (E), and late (L). The immunomodulatory genes m4, m6, and m152 are all E genes. An epitope from the IE1 gene product pp89 is recognized by a substantial number of CD8 T cells in BALB/c mice (12–14). Because pp89 is expressed before m4, m6, and m152, it is presumably less affected than E Ags are by these immunomodulatory genes. The pp89 Ag is presented if gene expression is limited to IE genes, but once E genes are expressed pp89 presentation is abolished due to the action of immune evasion genes (15). However, the addition of IFN-γ enables pp89-specific CTL to recognize infected cells even after E gene expression (16); in contrast, the E-specific CTL clones that we have isolated from C57BL/6 (B6) mice are not able to recognize wild-type MCMV-infected targets even with the addition of IFN-γ (3–5). The fact that inhibition of...
pp89 presentation is readily overcome may indicate that a significant amount of Ag presentation has already occurred before the immune evasion genes are expressed.

For many years, pp89 was the only MCMV CTL Ag identified, and in consequence it has been widely studied. However, most CTL in both BALB/c and B6 mice actually recognize E and not IE Ags (15, 17–19). Thus, paradoxically, the Ags that would seem most likely to be affected by immunomodulatory genes actually elicit the majority of the immunodominant responses. This led us to question what effect the viral immune evasion genes, specifically m152, which has the most profound effect, have on the immunodominance of E Ags. Until recently, no immunodominant E Ags for MCMV had been described. Three CTL epitopes from E genes (m4, M83, and M84) for BALB/c mice were identified by Holtappels and colleagues (20–22), but these are recognized by only a small number of CD8 T cells. Very recently, Holtappels et al. (23) identified a D\(^{b}\)-restricted epitope in m164, which is as immunodominant as pp89. There is at present no information on the effect of immunomodulatory genes on the immunodominance of these epitopes.

In the current paper we describe the use of CTL clones and a genomic expression library to identify a D\(^{b}\)-restricted epitope encoded by the MCMV E gene M45. These clones were isolated from mice infected with a mutant MCMV lacking m152 and recognize viruses lacking m152 but are unable to detect wild-type virus in \(^{3}C\) release assays. We compared the M45-specific CTL response in mice infected with MCMV with and without m152. Surprisingly, the percentage of CD8 T cells recognizing M45 was similar in mice infected with wild-type virus or with viruses lacking m152. These data indicate that while m152 affects M45 Ag presentation in vitro it does not affect the immunodominance of M45 in vivo.

**Materials and Methods**

**Construction of the MCMV DNA expression libraries**

Two MCMV expression libraries representing the MCMV genome were constructed using MCMV DNA that was originally cloned as HindIII fragments into pUC-based plasmids (known as plasmids A–P) (24). Library I was constructed using equimolar amounts of HindIII fragments A, G–K, and M–P (equivalent to 99,563 bp of the MCMV genome) purified by agarose gel electrophoresis of the respective HindIII-digested plasmids. Similarly, library II (equivalent to 106,408 bp of the MCMV genome) was constructed using equimolar amounts of HindIII-purified fragments B–D, F, and L. Fragment E DNA (22,749 bp) was not included in the libraries. For expression library construction, the purified HindIII fragments (1,066–33,141 bp) were further fragmented by partial restriction enzyme digestion using a combination of BclI, BglII, BamHI (library I), or Sau3A1 (library II) to generate BamHI compatible ends and ligated to BamHI cut pUC4 HisMax (Invitrogen, Carlsbad, CA). The pcDNA4 HisMax A, B, and C vectors allow expression of DNA in each reading frame by staggered insertions of DNA fragments downstream of a QBI SP163 translatable enhancer. Characterization of a subset of clones showed that insert frequencies were 92 (I) and 83% (II), and the average insert sizes were 1,130 (I) and 530 bp (II). Approximately 30,000 independent clones (library I) and 25,000 independent clones (library II) were arrayed in pools of 50 clones per pool. DNA for transfections was prepared using Qiagen 96 Turbo-Prep plates (Qiagen, Valencia, CA).

**Library screening**

The libraries were screened using K41 cells, an SV-40 derived as previously described in Ref. 3. A pool of 50 DNA clones from library II was confirmed to elicit a positive response by two D\(^{b}\)-restricted MCMV-specific CTL clones (clones 3 and 55). From the positive pool, individual bacterial clones were derived and DNA was prepared and screened as described above. Individual bacterial clones that stimulated the CTL clones were sequenced. DNA was confirmed to be MCMV sequence. Peptides were synthesized based on an algorithm of optimal D\(^{b}\) peptide-binding motifs (http://bimas.dcr.nih.gov/molbio/hla_bind).

**CTL line and clones**

Generation and maintenance of CTL clones 3 and 55 have been previously described (3). A polyclonal M45-specific CTL line was generated by methods previously described (23). Briefly, 1.5 × 10\(^{5}\) splenocytes from K\(^{b}\)-mice chronically infected with MCMV were incubated for 4 days with clone medium (RPMI with 10% FBS, 10^{-5} M 2-ME, and 10% rat IgA supernatant) and the M45 peptide at a concentration of 10^{-10} M. Recombinant human IL-2 (100 U) was added on day 4 and the medium was replaced with fresh clone medium. The cells were used 7 days after one round of stimulation with the M45 peptide.

**Viruses**

MCMV Smith was purchased from American Type Culture Collection (1399-VR). ΔMCMV 26 (ΔM152) and ΔMCMV 27 (wild-type rescue) (10), ΔM594.5 (lacking open reading frames m151–165) (9), BAC-derived wild-type virus MW97.01 (26), and ΔM152-MW99.05 (3) have been described.

**Mice**

Six-week-old female C57BL/6 mice were purchased from Simonsen Laboratories (Gilroy, CA), K\(^{b}\)-mice, the gift of F. Lemmonier (Institute Pasteur, Paris, France), were maintained in animal facilities at Oregon Health and Science University (Portland, OR) and were used in experiments earlier no 6 wk postbirth.

**CTL assays**

Either IFN-γ-pretreated, B6 mouse embryo fibroblasts (MEF) or untreated JAWS II cells (CRL-11904; American Type Culture Collection) were used as virus-infected targets. The addition of IFN-γ to JAWS II cells does not alter Ag presentation to MCMV-specific CD8\(^{+}\) T cells and therefore was not added to the cultures (M. C. Gold, unpublished data). Cells were infected with 50 PFU (except when noted) of virus for 16 h in the presence of 300 μg/ml phosphonoacetic acid (PAA; Sigma-Aldrich, St. Louis, MO) to prevent L gene expression. IFN-γ only or IFN-γ and E gene expression were enhanced as previously described (15). Briefly, to selectively enhance IFN-γ gene expression, B6 targets were infected with MCMV in the presence of cycloheximide (50 μg/ml; Sigma-Aldrich) followed by actinomycin D (5 μg/ml; Sigma-Aldrich). IFN-γ and E gene expression, cells were infected in the presence of cycloheximide alone followed by a 3-h incubation without drugs. For peptide screening, 10\(^{5}\) 51Cr-labeled RMA-S target cells were incubated with peptide at the concentrations indicated and plated with CTL clones at an E:T ratio of 15:1. After 5 h, the amount of radioactivity (cpm) in the supernatant was counted using a TopCount (Packard Instrument, Meriden, CT). The percentage of specific lysis was determined from the following equation: (cpm experimental release – cpm medium release)/(cpm total release – cpm medium release) × 100.

**ICS assay**

Splenocytes were isolated from MCMV-infected mice. APC (JAWS II cells) were infected with MCMV (ΔM152-MW99.05) at an MOI of 100 for 16 h in the presence of PAA. Effector splenocytes were incubated with peptide at various concentrations, or at a ratio of 1:1 with infected or uninfected JAWS II cells for 5 h in the presence of brefeldin A (GolgiPlug; BD PharMingen, Franklin Lakes, NJ). Cells were washed, incubated with FcBlock (BD PharMingen), and surface stained with an Ab to the CD8\(^{+}\) chain (BD PharMingen). Cells were then fixed and permeabilized using BD PharMingen’s Cytofix/Cytoperm kit before staining with an Ab to CD8\(^{+}\) (BD PharMingen). CD8\(^{+}\) T cells were analyzed by flow cytometry using FACS in conjunction with CellQuest software (BD PharMingen). All further analyses were performed using FlowJo software (Treestar, San Carlos, CA).
Materials and Methods

Two CTL clones (clones 3 and 55) were isolated from B6 mice infected with m152 virus lacking m152 and used intracellular cytokine staining (ICS) to determine how many hours of infection were required for Ag recognition by clone 3. The presence of brefeldin A in the ICS assay precludes further export of MHC class I in the infected cells and thus restricts the MHC-peptide complexes detected to those already at the cell surface at the indicated time postinfection. Fig. 1B shows that clone 3 detected Ag 6 h postinfection, and that recognition increased thereafter (Fig. 1B). This is well after m152 expression, which commences 2 h postinfection (5, 20). To confirm that the assay was capable of detecting Ag expression earlier in the infectious cycle we included for comparison another MCMV-specific CTL clone. Fig. 1B shows that clone 11 detected Ag by 4 h postinfection and showed quite different kinetics. Therefore, clone 3 detects an Ag expressed in the infectious cycle after m152 is active. We concluded that the Ag recognized by clone 3 would be a useful indicator with which to investigate the effect of the immunomodulatory genes on immunodominance.

D\(^\beta\)-restricted CTL clones recognize aa 985–93 of M45

From the MCMV genomic expression library, we identified a plasmid that both CTL clones 3 and 55 recognized. This plasmid encoded a small fragment of the M45 gene (28). Based on the published MCMV sequence (28) and the corrected addition of a cytotoxic cell line position 61918 of the MCMV genome (29), M45 encodes a 1174-aa protein that is homologous to human CMV UL45 and to the large subunit of the class I murine ribonuclease reductase gene (28).

From the translated sequence of the expressed M45 fragment, we predicted four potential D\(^\beta\)-binding peptides that were synthesized and used to sensitize RMA-S cells for lysis by clones 3 and 55 in a \(^{51}\)Cr release assay. Fig. 2 shows that both the 9-mer HGI-RNASFI (aa 985–993) and the 10-mer KHGIRNASFI (aa 984–993) were equally able to sensitize targets for killing by clones 3 and 55. Both peptides sensitized targets down to 10\(^{-12}\) M. As a measure of the relative affinity of HGI-RNASFI and KHGIRNASFI, we compared their ability to stabilize cell surface D\(^\beta\) in the TAP-deficient cell line, RMA-S (27). As shown in Fig. 3, the 9-mer and the 10-mer were equally effective at stabilizing cell surface D\(^\beta\). It is likely that the 9-mer represents the minimal epitope with the histidine residue occupying the A pocket of D\(^\beta\) (30).

Detection of M45\(_{985–993}\)-specific CD8s directly ex vivo

We next determined whether we could detect HGI-RNASFI-specific CD8 T cells directly ex vivo. Because of the low frequency of

allow E gene expression while blocking L gene expression indicated that clone 3 was specific for an E Ag (Fig. 1A). E gene expression occurs in waves, with m152 expression occurring in the earliest wave, 2 h postinfection (5, 20). We predicted that Ags expressed after m152 is expressed would be most susceptible to the effects of m152. To determine when in the infectious cycle the Ag recognized by clone 3 is expressed, we asked when it could be presented if m152 were absent. To this end, we infected cells with virus lacking m152 and used intracellular cytokine staining (ICS) to determine how many hours of infection were required for Ag recognition by clone 3. The presence of brefeldin A in the ICS assay precludes further export of MHC class I in the infected cells and thus restricts the MHC-peptide complexes detected to those already at the cell surface at the indicated time postinfection.

Fig. 2. Clones 3 and 55 recognize HGI-RNASFI and KHGIRNASFI peptides. Clones 3 and 55 were used as effectors in a \(^{51}\)Cr release assay using RMA-S target cells loaded with four peptides titrated from 10\(^{-7}\) to 10\(^{-13}\) M.
CD8s responding to previously identified E Ags, we optimized our chances of detecting (K)HGIRNASFI-specific CD8 T cells in vivo by looking at the peak of the response in mice whose only class I molecule is Dk. Kb−/− mice were infected with the MCMV mutant ΔMS94.5 (which lacks 14 genes, including m152). The ICS assay was used to quantify the frequency of M45-specific CD8+ spleen cells from acutely infected mice. Over 20% of all CD8+ splenocytes from Kb−/− mice infected for 6 days with ΔMS94.5 were (K)HGIRNASFI-specific (Fig. 4). We were encouraged by the strength of this response to look at the CD8 T cell response in chronically infected B6 mice. Fig. 4 shows the result from a representative mouse infected with MCMV-Δm152 for 1.5 years, in which 2.5% of CD8+ splenocytes recognized the (K)HGIRNASFI peptides (Fig. 4). Finally, no response to HGIRNASFI was detected in a naive B6 mouse, while in the same assay 4% of CD8s from a mouse infected with ΔMS94.5 for 12 wk made IFN-γ in response to HGIRNASFI (Fig. 5). In another experiment with four B6 mice chronically infected with viruses lacking m152, between 1 and 4% of CD8s were specific for HGIRNASFI, whereas <0.3% of CD8s were positive in two naive mice (data not shown). Other experiments suggest that the difference in the magnitude of the HGIRNASFI-specific responses seen in Fig. 4 is most likely to be due to differences between the acute and chronic phase of the CD8 response rather than a difference between Kb−/− mice and B6 mice. Future studies are planned to determine whether the HGIRNASFI-specific response changes in its relative proportion to the total MCMV-specific response over time in mice chronically infected with MCMV.

m152 ablates recognition of infected target cells by M45-specific CTL

The effects of the immunomodulatory gene m152 on M45 presentation in vitro are profound. Fig. 6A shows that clones 3 and 55 were unable to kill fibroblasts infected with three different wild-type MCMV strains. However, this defect was reversed if m152 was absent: clones 3 and 55 were able to kill fibroblasts infected with three different MCMV mutants lacking m152. m152 was equally effective at inhibiting presentation of M45 in MEFs and the dendritic cell (DC) line, JAWS II, that was used as the APC in the ICS assays. Fig. 6B shows a CTL assay using a polyclonal M45-specific CTL line: wild-type infected targets were not recognized, whereas Δm152-infected targets were readily lysed. We also tested this line for IFN-γ release by ICS, using both MEFs and JAWS II cells as APCs. Wild-type MCMV-infected MEFs and JAWS II cells failed to stimulate IFN-γ secretion, whereas both cell types infected with MCMV-Δm152 did stimulate IFN-γ secretion (data not shown). We conclude that m152 effectively inhibits presentation of HGIRNASFI in both MEFs and a DC line.

m152 does not affect the immunodominance of M45 in vivo

Because m152 appeared able to completely prevent presentation of M45, we hypothesized that mice infected with a wild-type (m152+) MCMV would not develop an M45-specific CTL response. To test this prediction, we infected Kb−/− mice with wild-type MCMV (Smith), ΔMS94.5, ΔMC96.24 (Δm152), and the rescued virus MC96.27 (ΔMC96.24 with m152 restored). Fig. 7 shows that, surprisingly, the percentage of CD8s recognizing M45 was very similar in each infection: between 12 and 13% of CD8+ splenocytes on day 8 postinfection were specific for M45.

Although mice infected with wild-type virus made a response to M45, we reasoned that m152 might nevertheless affect the immunodominance of M45 within the hierarchy of the total CD8 response to MCMV. We were unable to test this hypothesis by comparing the M45 response to other specific peptides because no other H-2k-restricted epitopes have yet been identified. Instead, we...
calculated the ratio of the M45-specific response to the response we could detect to the whole virus, which was assessed using virus-infected APCs in the ICS assay. This time we used B6 mice, where Db-HGIRNASFI also competes with K\textsuperscript{b}-restricted responses for immunodominance. Five mice were infected with the MCMV mutant /H9004\textsubscript{m152} (MC96.24) or the revertant (rMC96.27) MCMV strains for 8 days. Fig. 8 shows that CD8 T cell responses to MCMV (/H9004\textsubscript{m152})-infected DCs ranged from 3 to 11%. No remarkable differences were seen in the CD8 T cell responses to virus-infected APC after MCMV infection with or without m152. This indicated that m152 did not affect the total numbers of MCMV-specific CD8 T cells that were generated in vivo. This assay also allowed a rough assessment of the immunodominance of M45 within the total MCMV-specific CD8 response. While the HGIRNASFI peptide stimulation probably detects all CD8s of this specificity, it is likely that simulating with virus-infected DCs underestimates the total number of MCMV-specific CD8s. Therefore, we conclude that, while a substantial number of CD8s recognized HGIRNASFI, other specificities exist. Finally, we compared the HGIRNASFI-specific response to the detectable response to whole virus after infection with MCMV with or without m152. Although there was mouse to mouse variation, the ratio of the peptide-specific response to the total MCMV-specific response did not differ significantly (p = 0.39). The ratio of the M45-specific CD8 T cell response to the total MCMV-specific CD8 response averaged among five mice (expressed as a percentage) was 62.1% after MCMV\textsubscript{m152} infection vs 72.8% after infection with the revertant (Fig. 8 and Table I). These data again indicate that m152 did not alter the immunodominance of the M45 Ag.

**Discussion**

**Identification of an immunodominant MCMV CTL Ag in B6 mice**

In this paper we describe the use of CTL clones and a genomic fragment library to identify an immunodominant epitope in MCMV. Identification of CTL epitopes in large viruses remains a difficult procedure, and several points in the method described here are worthy of note. To initially isolate the CTL clones we screened

![Figure 6](http://www.jimmunol.org/)

**Figure 6.** A, m152 prevents Ag recognition by clones 3 and 55. Clones 3 and 55 were tested in a CTL assay with three different wild-type MCMV strains or with three mutants lacking m152; only the mutants were recognized. These results are typical of multiple experiments. B, m152 interferes with the ability of an M45-driven CTL line to kill both MEFs and JAWS II target cells infected with MCMV. M45-specific effectors were splenocytes from K\textsuperscript{b}/-/- mice infected with wild-type MCMV stimulated in vitro with 10\textsuperscript{-8} M HGIRNASFI peptide for 1 wk. Target cells were either B6 MEFs or JAWS II cells infected as indicated. The reduced killing on wild-type-infected targets compared with the killing seen on uninfected targets is frequently seen using polyclonal CTL and may reflect NK activity. A duplicate of this experiment showed similar results.

![Figure 7](http://www.jimmunol.org/)

**Figure 7.** m152 does not affect the M45-specific response in acutely infected K\textsuperscript{b}/-/- mice. Shown is quantification by ICS assay of HGIRNASFI-specific CD8\textsuperscript{+} splenocytes from K\textsuperscript{b}/-/- mice infected for 8 days with wild-type (Smith), ΔMS94.5, ΔMC96.24 (Δm152), and rMC96.27 (revertant) viruses. The number in the corner indicates the percentage of CD8 T cells specific for M45. Similar results were seen in two separate experiments with two mice per group.

![Figure 8](http://www.jimmunol.org/)

**Figure 8.** m152 does not affect the immunodominance of M45 in acutely infected B6 mice. HGIRNASFI-specific and total MCMV-specific responses were compared by ICS assay. Splenocytes from B6 mice infected for 8 days with Δm152 (ΔMC96.24) or revertant (rMC96.27) were incubated for 6 h either with 10\textsuperscript{-8} M HGIRNASFI peptide or with Δm152-infected JAWS II cells used as APCs. Data are representative of three experiments with a minimum of two mice per group.
clones for their ability to kill targets infected with a virus (∆MS94.5) that lacks the key immune evasion gene, m152. The CTL clones used in this paper were generated from ∆m152-infected mice, but we have also generated clones from wild-type-infected mice (3); CTL clones from both types of infection were able to lyse ∆MS94.5-infected targets but not wild-type-infected targets. Thus, the use of a virus lacking immune evasion genes to screen CTL clones was critical for isolation of these clones. M45 is immunodominant in wild-type infection, but clones of this specificity would not have been identified had we used wild-type virus in our initial screen. The use of viruses lacking immune evasion genes for screening clones may also be useful in identifying new CTL epitopes in other viruses such as human CMV, HSV, and Kaposi’s sarcoma-associated herpesvirus.

The second feature of the strategy described here was the use of a viral genomic fragment library. This strategy has previously been applied successfully to identify epitopes in Chlamydia and HSV (S. P. Fling, unpublished results). If a pathogen’s genomic DNA can be obtained independently of the host genome this approach can be useful, because it eliminates contaminating host transcripts that would be expressed in a cDNA library and which would increase the clonal complexity of the library. Another advantage of expression cloning in general is the potential to identify both dominant and subdominant epitopes. We do not yet know whether genomic libraries constructed by restriction digest express all possible epitopes. Clearly, if a restriction site coincides with the portion of DNA encoding an epitope, representation may be limited. However, this problem is abrogated to some extent by partial restriction digests of input DNA.

The M45 epitope is clearly recognized by a substantial fraction of MCMV-specific CD8s in B6 mice. Identification of other epitopes is necessary before we can determine whether it is the most immunodominant epitope in this strain. The only other immunodominant MCMV epitopes identified so far are for the H-2d haplotype: epitopes from pp89 (Ld-restricted) (12, 13) and m164 (H-9004). Very recently, Ye et al. (14) used a protocol similar to the one we used in the current paper and determined that pp89-specific CD8s made up close to 3% of CD8s 1 mo after infection. We measured a similar response to pp89 (4% of CD8s) in one experiment in BALB/c mice (M. C. Gold, unpublished observation). We think it more likely that differences in infectious route and method of detecting the response, rather than between BALB/c and B6 mice per se, will be found to account for these differences in the measured size of the response. It will be interesting to perform side by side comparisons using the same methods to determine the relative size of the overall MCMV-specific response in both mouse strains, as well as the proportion of that response that can be attributed to the currently identified epitopes.

m152 affects presentation of M45 without affecting its immunodominance

The hypothesis that m152 would affect the hierarchy of immunodominant CD8 epitopes is based on the fact that m152 severely impairs Ag processing and presentation in fibroblasts. However, our work demonstrates that the CD8 T cell response to M45 is not affected by the presence of m152 in vivo even though its presentation is severely affected by m152 in vitro. We can think of two possible explanations for this surprising result. First, Ag presentation in some important cell types in vivo, such as macrophages or DCs, may not be affected by m152 in the same way that fibroblasts are affected in vitro. It has been reported that macrophages can overcome the effect of immune evasion genes and effectively present the IE pp89 Ag (31). However, our experience is that M45-specific CTL clones are unable to kill wild-type virus-infected macrophages, even though they readily kill ∆m152-infected macrophages (D. LoPiccolo, M. Gold, D. Kavanagh, M. Wagner, U. Koszinowski, and A. Hill, manuscript in preparation). Similarly, we show in this paper that m152 effectively prevents Ag presentation in a DC line. Thus, while it remains possible that presentation of M45 by an infected cell in vivo is not affected by m152, there is currently no evidence to support that hypothesis.

A second explanation for the lack of effect of m152 on the immunodominance of M45 could be that cross-presentation is the principle mechanism for priming the CD8 T cell population to M45 in vivo. Cross-presentation is the process by which DCs take up Ag from infected cells and present it on newly synthesized class I molecules. Presumably m152, a glycoprotein that acts in the ER, only affects Ag presentation in infected cells and does not affect cross-presentation. Cross-presentation is believed to be an important mechanism by which CD8 T cells are primed in vivo (32, 33), and it is thus perhaps not surprising that M45-specific CD8 T cell responses would be elicited in wild-type MCMV-infected mice. Nevertheless, if cross-presentation is the main mechanism used for priming the CTL response, this could potentially be problematic for the immune response. If the Ags presented by cross-priming are different from those presented directly by virus-infected cells, then the immune response may be fooled into making a CTL response that is ineffective in clearing virus-infected cells. Indeed, we had assumed the CD8 T cell response would focus on Ags that are efficiently presented by virus-infected cells in vivo. In an attempt to reduce in vitro artifact, we assessed the efficacy of immune evasion genes using virus infections (rather than transfusions) of primary untransformed murine fibroblasts, as well as a DC line. In these assays m152 prevented recognition of the M45-encoded epitope. However, we do not yet know how effective M45-specific CD8s are in vivo against wild-type virus or, indeed, for which infected cell types in vivo CD8 control is most important. However, we do note that MCMV is effectively controlled by the immune response and that CD8 T cells play an important role in this control. Future studies will address these issues.
References