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The Mouse Cytomegalovirus Glycoprotein m155 Inhibits CD40 Expression and Restricts CD4 T Cell Responses▽

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Cytomegaloviruses (CMV) utilize a variety of immunomodulatory strategies to facilitate the establishment of lifelong persistence in their infected hosts. We show that the mouse CMV (MCMV) m155 open reading frame (ORF) is required for the posttranscriptional inhibition of CD40 expression in infected antigen-presenting cells. Consistent with the known importance of CD40-mediated costimulation of T cells, a m155-deficient virus induces enhanced MCMV epitope-specific CD4 T cell responses.

Healthy individuals infected with human cytomegalovirus (HCMV) are largely asymptomatic, but HCMV infection can result in high morbidity and death in immunosuppressed patients (12, 38, 48). HCMV establishes lifelong persistence, which is thought to require manipulation of the host's immune system by the coordinated function of many viral immunomodulatory genes (34).

As adaptive immune responses develop during infection, interactions occur between mature antigen-presenting cells (APC) and their cognate T cells. The quality and quantity of the resulting T cell responses are regulated in large part by the affinity of peptide/major histocompatibility complex-T cell receptor (MHC-TCR) interactions and commensurate cosignals mediated by members of the tumor necrosis factor receptor (TNFR) and CD28 families. CD40 is a costimulatory TNFR superfamily member that is upregulated upon APC maturation and plays a role in promoting both CD4 and CD8 T cell responses (reviewed in references 14 and 29). The infection of dendritic cells with mouse CMV (MCMV) or HCMV restricts antigen presentation, inhibits costimulatory molecule expression, and enhances the expression of negative cosignaling ligands, resulting in reduced priming, expansion, and survival of antigen-specific T cells that encounter these APC (3, 7, 8, 16, 17, 19, 22, 35). Consequently, it is quite possible that these mechanisms contribute to the immune suppression that is observed for cases of clinical HCMV disease (10, 15, 44).

MCMV infection results in reduced cell surface expression of CD40 in dendritic cells (DC) (3, 8). Two cell lines were utilized to investigate this effect; the RAW264.7 monocytic macrophage cell line and the DC2.4 bone marrow-derived DC line (kindly provided by K. Rock) (41). Throughout the study, fluorescence-activated cell sorter (FACS) and immunofluorescence experiments with MCMV that expresses green fluorescent protein (MCMV-GFP) (33) were performed using multiplicities of infection (MOI) of 0.4 and centrifugal enhancement, resulting in approximately 30% infected RAW264.7 and 20% infected DC2.4 cells, respectively. Immunoblot and RNA analysis of infected cells used MOI of 1.2 and centrifugal enhancement. Microscopy of infected and uninfected cells 24 h postinfection (p.i.), with staining at 4°C using biotinylated anti-CD40 (Becton Dickinson, Heidelberg, Germany) and Alexa Fluor 546-conjugated streptavidin (Invitrogen, Karlsruhe, Germany), confirmed that wild-type MCMV-GFP downregulates CD40 surface expression (Fig. 1B).

To identify the responsible MCMV open reading frame (ORF), previously described MCMV-GFP deletion mutants generated by homologous recombination with the help of bacteriophage λ recombination genes redα, β, and γ (20, 28, 33) were utilized (Fig. 1A). Both cell lines were treated with 500 U/ml gamma interferon (IFN-γ; Immunotools, Friesoythe, Germany) and surface CD40 or CD86 expression at 24 h p.i. was analyzed by flow cytometry, using biotinylated antibodies and streptavidin-allophycocyanin or streptavidin-phycoerythrin (Becton Dickinson) for detection (Fig. 1C). In cells infected with MCMV-GFPΔ6, CD40 expression was unaffected (Fig. 1C, MCMV-GFPΔ6), indicating a role for one or more of the ORFs encompassed in the deleted region (m144 to m158). Subsequent infection with MCMV-GFPΔm155 (20) (nucleotides [nt] 214440 to 215476 deleted) (37) revealed this single ORF to be necessary for the observed phenotype (Fig. 1C, MCMV-GFPΔm155). To confirm that m155 is required to restrict CD40 expression, the m155 ORF (nucleotides 214360 to 215575) (37) was inserted into the MCMV-GFPΔ6 genome under the control of the HCMV major immediate early promoter, as previously described (20). Infection with this "revertant" virus (MCMV-GFPm155ins) resulted in CD40 downregulation, verifying the requirement for m155 (Fig. 1C, MCMV-GFPm155ins). Expression of the costimulatory molecule CD86 is inhibited by the m147.5 ORF located within the Δ6 region but was unaffected in MCMV-GFPm155ins-infected cells (Fig. 1D, MCMV-GFPm155ins), confirming that m155 does not disrupt general cell surface protein expression (28).

m155 is a member of the MCMV m145 family of 10 predicted membrane glycoproteins (37). Eight m145 family members, including m155, have predicted or proven structural ho-

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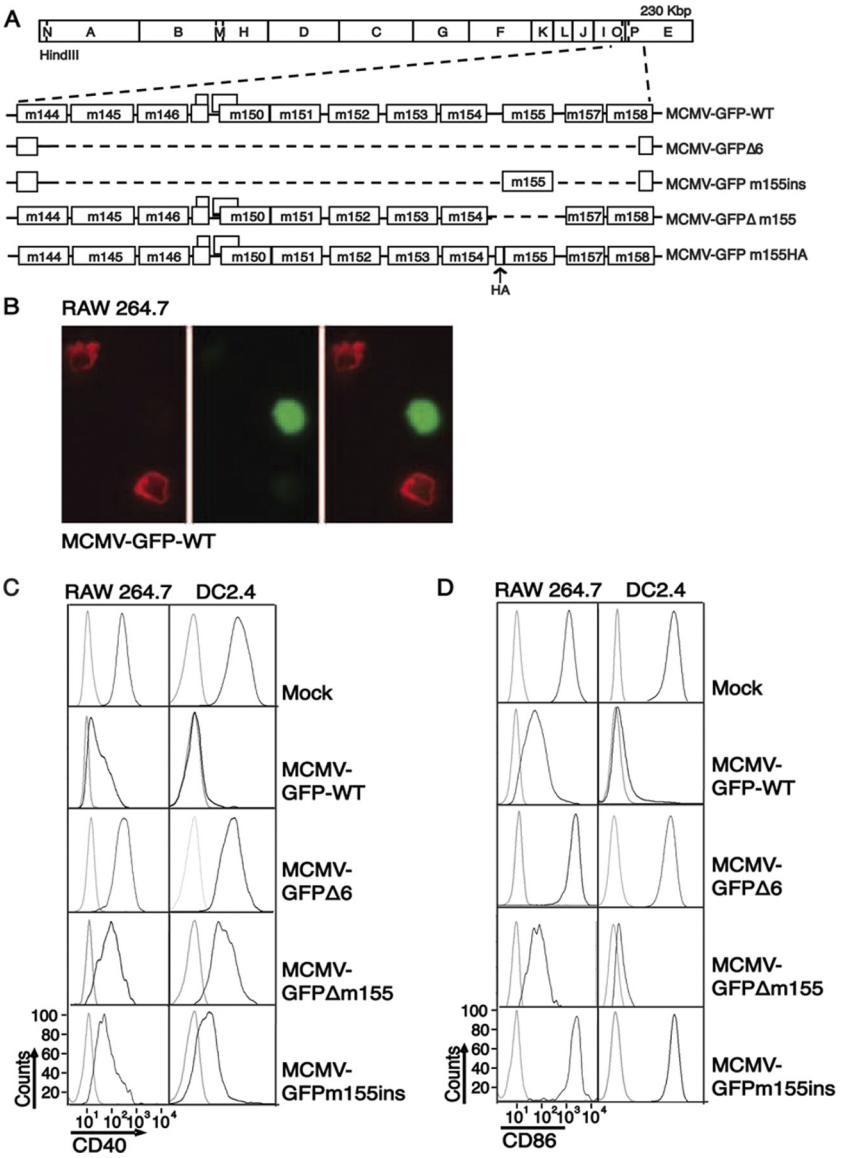

FIG. 1. (A) Schematic depiction of MCMV mutants utilized in this study. MCMV-GFPm155 was designed based on the MCMV-Δ6 backbone inserting the m155 gene under the control of the HCMV major immediate early promoter. Boxes, indicated ORFs; dashed lines, deleted areas replaced by kanamycin resistance gene; solid lines, distance between ORFs. WT, wild type. (B) RAW264.7 cells were infected with MCMV-GFP, and CD40 surface expression was analyzed at 24 h p.i. by microscopy. Red, CD40; green, MCMV-derived GFP. (C and D) RAW264.7 or DC2.4 cells were infected with the indicated viruses. CD40 or CD86 surface expression was determined at 18 h p.i. Black lines, indicated antibody; gray lines, isotype control. Gated for GFP^+ (MCMV-GFP-infected) cells.

mology to MHC class I molecules (2, 31, 42). This structure underlies the interactions of four m145 family proteins (m145, m152, m157, and m155) with MHC class I or NK cell ligands that contain MHC class I structural elements (2, 5, 20, 24, 26, 27, 42, 50). The downregulation of CD40 by m155 is the first example of an m145 family member targeting a protein unrelated to MHC class I, suggesting a novel recognition mechanism. The second known function of m155, downregulation of H60 (20, 27), is shared with m138 (25), an immunoevasin that also restricts expression of two other NK cell ligands (4, 24, 25). Consequently, the emerging picture depicts a complex network of multifunctional CMV immunomodulatory proteins that shape the antiviral immune response.

Transcription of m155 in MCMV-infected RAW264.7 cells was analyzed 24 h p.i. by Northern blotting, using 5 μg of cytoplasmic mRNA and a [α-^{32}P]dCTP-labeled probe comprising the m155 ORF (nt 214444 to 215536) generated using a random primer labeling kit (Amersham, Freiburg, Germany). A 2.1-kb mRNA transcript was detected (Fig. 2A), similar to that previously observed for MCMV-infected NIH 3T3 cells (1, 49). To examine m155 protein expression, an influenza hemagglutinin (HA) epitope tag was inserted at the C-terminal end of the m155 ORF in the bacterial artificial chromosome (BAC) genome of the MCMV-GFP virus as previously described (28). Lysates of cells infected with the m155-HA virus were then analyzed by SDS-PAGE and subsequently immunoblotted us-

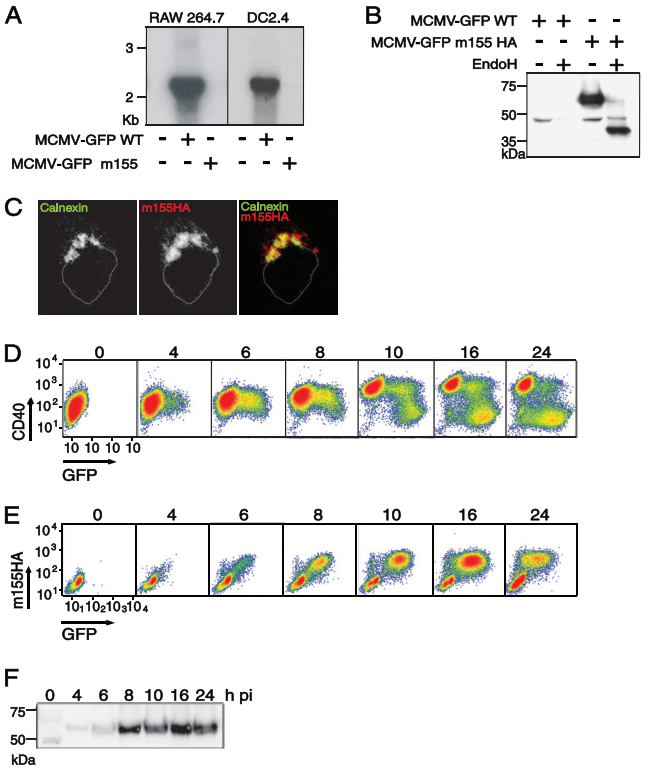

FIG. 2. Characterization of m155 expression and its correlation with CD40 downregulation. (A) RAW264.7 or DC2.4 cells were infected with the indicated viruses and RNA corresponding to the m155 transcript identified by Northern blotting. (B) RAW264.7 cells were infected as indicated, and HA-tagged proteins were detected by immunoblotting cell lysates. Lysates were treated with endoglycosidase H (EndoH) where shown. + or −, with or without indicated virus or EndoH. (C) DC2.4 cells were infected with MCMV m155-HA and analyzed 10 h p.i. by confocal microscopy. m155-HA and the ER marker calnexin are shown. Cell nuclei were detected using Hoechst 33342 DNA stain and are indicated. The overlap between m155-HA (red) and calnexin (green) is shown in yellow. (D) RAW264.7 cells were infected with MCMV-GFP wild type, and CD40 surface expression was measured at the indicated times postinfection. (E) RAW264.7 cells were infected with MCMV m155-HA, and intracellular m155-HA expression was measured as indicated. (F) Lysates of RAW264.7 cells infected with MCMV-GFPm155HA for the times shown were analyzed by immunoblotting.

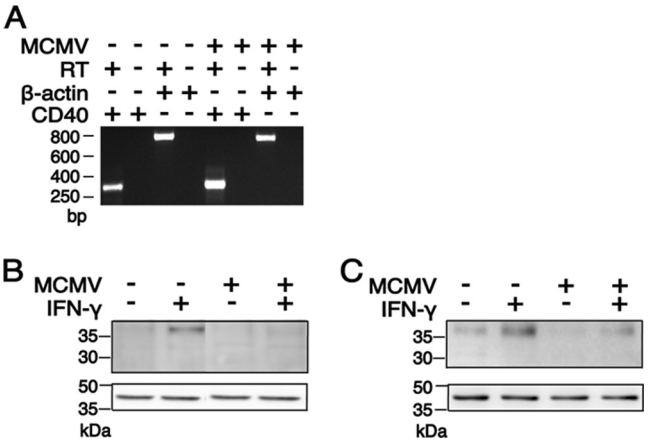

FIG. 3. CD40 downregulation results from a loss of protein expression. (A) RAW264.7 cells were infected with the MCMV-GFP wild type or were left uninfected, and cytoplasmic RNA was extracted and analyzed for the presence of CD40 and actin transcripts. +, reverse transcriptase (RT) treated; −, without reverse transcriptase. RAW264.7 (B) and DC 2.4 (C) cells were mock infected or infected with MCMV-GFP, and CD40 (top) and β-actin (bottom) proteins were detected by immunoblotting.

ing a primary rabbit anti-HA antibody and a secondary horseradish peroxidase (HRP)-conjugated anti-rabbit antibody (Sigma, Munich, Germany) (Fig. 2B). While the predicted size of m155 is 43 kDa, m155-HA from infected cell lysates migrated with a molecular mass of ~60 kDa by this analysis. When cell lysates were treated with 0.025 U endoglycosidase H (Roche Diagnostics, Mannheim, Germany) at 37°C for 16 h in 50 mM sodium citrate buffer (pH 5.5), m155-HA was detected at the predicted size of ~43 kDa, indicating that this protein is modified by N-linked glycosylation (Fig. 2B). Interestingly, all detectable m155-HA protein was susceptible to endoglycosidase H treatment, suggesting that the protein does not exit the endoplasmic reticulum (ER)/*cis*-Golgi compartment. Immunofluorescence analysis of MCMV m155-HA-infected DC2.4 cells 10 h p.i. was then performed (Fig. 2C), using a primary rabbit anti-HA antibody (Sigma) and a secondary Alexa 568-conjugated anti-rabbit antibody (Invitrogen) to detect the m155-HA protein (middle panel), and a mouse anti-calnexin antibody (Abcam, Cambridge, United Kingdom) with a secondary Cy5-conjugated anti-mouse antibody (Jackson ImmunoResearch, Suffolk, United Kingdom) as an ER marker (left panel). This analysis confirmed that most of the m155-HA protein localizes to the ER, in agreement with the predominantly intracellular localization described for m155 in MCMV-infected COS-7 cells (right panel) (32). This contrasts with the observed localization of m155 in CV-1 cells when transiently expressed in the absence of MCMV infection, suggesting that an additional viral or cellular protein(s) may be required to direct m155 to the ER (20).

To confirm a temporal correlation between m155-HA protein expression and CD40 downregulation, infected RAW264.7 cells were analyzed in parallel at several time points by surface and intracellular FACS (Fig. 2D and E) and immunoblotting (Fig. 2F). Intracellular m155-HA expression was detected using rabbit anti-HA (Sigma) and a phycoerythrin-conjugated anti-rabbit secondary antibody (eBioscience, Frankfurt, Germany). m155-HA expression correlated with the observed timing of CD40 downregulation.

To further characterize the mechanism of m155-mediated CD40 downregulation, the level of CD40 mRNA was analyzed by semiquantitative PCR analysis of cDNA isolated from RAW264.7 cells infected with MCMV-GFP at 24 h p.i. (Fig. 3A), using the following primers: CD40 sense, 5′-CTG CCC AGT CGG CTT CTT CTC-3′; CD40 antisense, 5′-CCT GTG TGA CAG GCT GAC AC-3′; actin sense, 5′-CAG AGG CTC CCC TAA ATC CC-3′; and actin antisense, 5′-CAC ACT GAG TAC TTG CGC TC-3′. MCMV-GFP infection did not result in a reduction of the CD40 transcript, indicating that downregulation of this costimulatory receptor by m155 occurs posttranscriptionally.

Next, CD40 protein levels in MCMV-GFP-infected cell lysates were analyzed by immunoblotting, using rabbit anti-

CD40 (Stressgen, Aachen, Germany) and anti-actin (Sigma) antibodies and a secondary HRP-conjugated anti-rabbit antibody for detection. CD40 protein expression was undetectable in RAW264.7 (Fig. 3B) or DC2.1 cells (Fig. 3C) after MCMV-GFP infection, regardless of whether cells were treated with IFN-γ or not. The restriction of CD40 cell surface expression therefore appears to be due to a reduction in total cellular CD40 protein. Treatment of infected cells with proteasomal and lysosomal protease inhibitors (MG132 and leupeptin, respectively) had no discernible effect on the loss of CD40 (data not shown). Cleavage of CD40 at the plasma membrane to release a soluble extracellular form of the protein has been reported for human B cell lines (11) but also could not be detected upon MCMV infection (data not shown). The mechanism of CD40 loss from MCMV-infected cells therefore remains unknown and is the subject of further investigations.

m155 functions as a virulence factor in mice; MCMV mutants lacking m155 cause reduced pathogenicity in BALB/c SCID mice and are controlled more readily by NK cells in immunocompetent BALB/c mice due to their inability to restrict H60 expression (1, 20, 27). The lack of H60 in B6 mice (13, 26, 30) allows for the study of possible effects of m155 on adaptive immune responses in the absence of its NK cell modulatory capacity. To accomplish this, an m155 deletion virus was constructed as previously described (9) using the BAC-cloned genome of the K181 strain of MCMV (kindly provided by A. Redwood) (40).

As CD4 and CD8 T cells differ in their requirements for APC-derived costimulation, we examined MCMV epitope-specific responses of both T cell types in the spleen 8 days postinfection with wild-type or m155-deletion viruses (6, 36). Splenocytes were restimulated *in vitro* with purified peptide epitopes for CD8 or CD4 T cells as previously published (6) and IFN-γ- or IFN-γ/TNF-secreting cells were analyzed by flow cytometry. Examination of $m38_{8-15}$-, $m45_{985-993}$-, and $m139_{419-426}$ (23) epitope-specific CD8 T cell responses revealed there to be no statistically significant differences in mice infected with wild-type or MCMVΔm155 viruses (Fig. 4A). Significantly higher percentages of $m25_{409-423}$ epitope-specific IFN-γ- and IFN-γ/TNF-producing CD4 T cells were seen for mice infected with MCMVΔm155 than in those infected with wild-type MCMV (Fig. 4B). These results indicate that the absence of the m155 ORF has an enhancing effect on MCMV-specific CD4 T cell responses in B6 mice at early times postinfection.

Both human and mouse CMV have been previously shown to limit cytokine production by CD4 T cells in DC-T cell culture systems, which has been postulated to be due to reduced expression of MHC class II (21, 39). The quality of the CD4 T cell response is CD40 dependent in lymphocytic choriomeningitis virus (LCMV) and adenovirus models; CD4 T cell priming is restricted in the absence of interactions between CD40 on APC and CD40L on activated CD4 T cells, and CD4 help-dependent CD8 T cell responses, such as MCMV IE3 responses, are similarly dependent on the CD40/CD40L interaction (18, 43, 45–47).

A possible explanation for the observed inhibition of CD4 T cell expansion by m155 is that downregulation of CD40 results in insufficiently stimulated APC that are unable to prime normal CD4 T cell responses. Although further analysis is required to confirm whether this is the precise reason for the observed reduction in functional CD4 T cell responses, we have identified the m155 protein as a participant in CD4 T cell regulation and as the first m145 family protein known to target a member of the TNFR family.

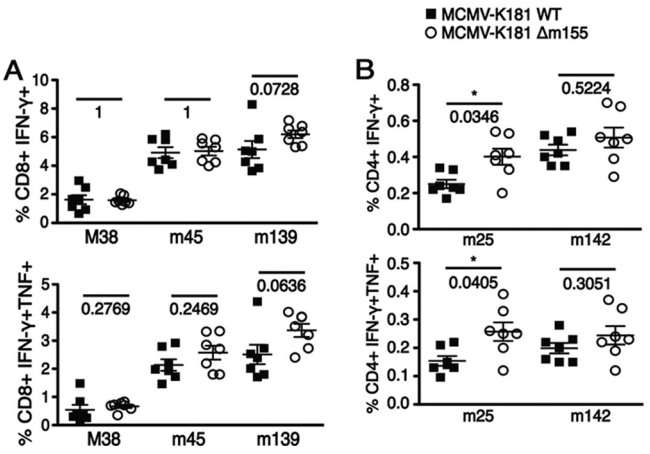

FIG. 4. MCMV-specific CD4 T cell frequency increases in the absence of m155. (A) Splenocytes from mice infected with wild-type (black squares) or m155 deletion (white circles) viruses were restimulated with the indicated peptides at 8 days p.i., and CD8 (A) or CD4 (B) T cell cytokine production was analyzed by intracellular cytokine staining. Graphs represent the percentage of gated splenic CD4 T cells producing IFN-γ (left panel) and IFN-γ and TNF (right panel). Representative results from two experiments are shown. Statistical significance was determined with the two-tailed Mann-Whitney test. Asterisks indicate significance ($P < 0.05$); numbers under vertical lines are P values.

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REFERENCES

1. **Abenes, G., et al.** 2004. Murine cytomegalovirus with a transposon insertional mutation at open reading frame m155 is deficient in growth and virulence in mice. J. Virol. **78:**6891–6899.
2. **Adams, E. J., et al.** 2007. Structural elucidation of the m157 mouse cytomegalovirus ligand for Ly49 natural killer cell receptors. Proc. Natl. Acad. Sci. U. S. A. **104:**10128–10133.
3. **Andrews, D. M., C. E. Andoniou, F. Granucci, P. Ricciardi-Castagnoli, and M. A. Degli-Esposti.** 2001. Infection of dendritic cells by murine cytomegalovirus induces functional paralysis. Nat. Immunol. **2:**1077–1084.
4. **Arapovic, J., R. T. Lenac, A. B. Reddy, A. Krmpotic, and S. Jonjic.** 2009. Promiscuity of MCMV immunoevasin of NKG2D: m138/fcr-1 down-modulates RAE-1epsilon in addition to MULT-1 and H60. Mol. Immunol. **47:** 114–122.
5. **Arase, H., E. S. Mocarski, A. E. Campbell, A. B. Hill, and L. L. Lanier.** 2002. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. Science **296:**1323–1326.
6. **Arens, R., et al.** 2008. Cutting edge: murine cytomegalovirus induces a polyfunctional CD4 T cell response. J. Immunol. **180:**6472–6476.
7. **Beck, K., U. Meyer-Konig, M. Weidmann, C. Nern, and F. T. Hufert.** 2003. Human cytomegalovirus impairs dendritic cell function: a novel mechanism of human cytomegalovirus immune escape. Eur. J. Immunol. **33:**1528–1538.
8. **Benedict, C. A., A. Loewendorf, Z. Garcia, B. R. Blazar, and E. M. Janssen.** 2008. Dendritic cell programming by cytomegalovirus stunts naive T cell responses via the PD-L1/PD-1 pathway. J. Immunol. **180:**4836–4847.
9. **Borst, E. M., C. Benkartek, and M. Messerle.** 2007. Use of bacterial artificial chromosomes in generating targeted mutations in human and mouse cytomegaloviruses. Curr. Protoc. Immunol. **Chapter 10:**Unit 10.32.
10. **Carney, W. P., and M. S. Hirsch.** 1981. Mechanisms of immunosuppression

in cytomegalovirus mononucleosis. II. Virus-monocyte interactions. J. Infect. Dis. **144:**47–54.
11. **Contin, C., et al.** 2003. Membrane-anchored CD40 is processed by the tumor necrosis factor-alpha-converting enzyme: implications for CD40 signaling. J. Biol. Chem. **278:**32801–32809.
12. **Crough, T., and R. Khanna.** 2009. Immunobiology of human cytomegalovirus: from bench to bedside. Clin. Microbiol. Rev. **22:**76–98.
13. **Diefenbach, A., A. M. Jamieson, S. D. Liu, N. Shastri, and D. H. Raulet.** 2000. Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. Nat. Immunol. **1:**119–126.
14. **Elgueta, R., et al.** 2009. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol. Rev. **229:**152–172.
15. **Frascaroli, G., et al.** 2006. Dendritic cell function in cytomegalovirus-infected patients with mononucleosis. J. Leukoc. Biol. **79:**932–940.
16. **Gredmark, S., and C. Soderberg-Naucler.** 2003. Human cytomegalovirus inhibits differentiation of monocytes into dendritic cells with the consequence of depressed immunological functions. J. Virol. **77:**10943–10956.
17. **Gredmark, S., T. Tilburgs, and C. Soderberg-Naucler.** 2004. Human cytomegalovirus inhibits cytokine-induced macrophage differentiation. J. Virol. **78:**10378–10389.
18. **Grewal, I. S., J. Xu, and R. A. Flavell.** 1995. Impairment of antigen-specific T-cell priming in mice lacking CD40 ligand. Nature **378:**617–620.
19. **Grigoleit, U., et al.** 2002. Human cytomegalovirus induces a direct inhibitory effect on antigen presentation by monocyte-derived immature dendritic cells. Br. J. Haematol. **119:**189–198.
20. **Hasan, M., et al.** 2005. Selective down-regulation of the NKG2D ligand H60 by mouse cytomegalovirus m155 glycoprotein. J. Virol. **79:**2920–2930.
21. **Heise, M. T., M. Connick, and H. W. Virgin.** 1998. Murine cytomegalovirus inhibits interferon gamma-induced antigen presentation to CD4 T cells by macrophages via regulation of expression of major histocompatibility complex class II-associated genes. J. Exp. Med. **187:**1037–1046.
22. **Hertel, L., V. G. Lacaille, H. Strobl, E. D. Mellins, and E. S. Mocarski.** 2003. Susceptibility of immature and mature Langerhans cell-type dendritic cells to infection and immunomodulation by human cytomegalovirus. J. Virol. **77:**7563–7574.
23. **Humphreys, I. R., et al.** 2007. OX40 costimulation promotes persistence of cytomegalovirus-specific CD8 T Cells: a CD4-dependent mechanism. J. Immunol. **179:**2195–2202.
24. **Krmpotic, A., et al.** 2005. NK cell activation through the NKG2D ligand MULT-1 is selectively prevented by the glycoprotein encoded by mouse cytomegalovirus gene m145. J. Exp. Med. **201:**211–220.
25. **Lenac, T., et al.** 2006. The herpesviral Fc receptor fcr-1 down-regulates the NKG2D ligands MULT-1 and H60. J. Exp. Med. **203:**1843–1850.
26. **Lodoen, M., et al.** 2003. NKG2D-mediated natural killer cell protection against cytomegalovirus is impaired by viral gp40 modulation of retinoic acid early inducible 1 gene molecules. J. Exp. Med. **197:**1245–1253.
27. **Lodoen, M. B., et al.** 2004. The cytomegalovirus m155 gene product subverts natural killer cell antiviral protection by disruption of H60-NKG2D interactions. J. Exp. Med. **200:**1075–1081.
28. **Loewendorf, A., et al.** 2004. Identification of a mouse cytomegalovirus gene selectively targeting CD86 expression on antigen-presenting cells. J. Virol. **78:**13062–13071.
29. **Ma, D. Y., and E. A. Clark.** 2009. The role of CD40 and CD154/CD40L in dendritic cells. Semin. Immunol. **21:**265–272.
30. **Malarkannan, S., et al.** 1998. The molecular and functional characterization of a dominant minor H antigen, H60. J. Immunol. **161:**3501–3509.
31. **Mans, J., et al.** 2007. Cellular expression and crystal structure of the murine cytomegalovirus major histocompatibility complex class I-like glycoprotein, m153. J. Biol. Chem. **282:**35247–35258.
32. **Mans, J., et al.** 2009. Structure and function of murine cytomegalovirus MHC-I-like molecules: how the virus turned the host defense to its advantage. Immunol. Res. **43:**264–279.
33. **Mathys, S., et al.** 2003. Dendritic cells under influence of mouse cytomegalovirus have a physiologic dual role: to initiate and to restrict T cell activation. J. Infect. Dis. **187:**988–999.
34. **Mocarski, E. S., T. Shenk, and R. F. Pass.** 2007. Cytomegaloviruses, p. 2701–2772. *In* D. M. Knipe, et al. Fields virology, 5th ed. Lippincott Williams & Wilkins, Philadelphia, PA.
35. **Moutaftsi, M., A. M. Mehl, L. K. Borysiewicz, and Z. Tabi.** 2002. Human cytomegalovirus inhibits maturation and impairs function of monocyte-derived dendritic cells. Blood **99:**2913–2921.
36. **Munks, M. W., et al.** 2006. Genome-wide analysis reveals a highly diverse CD8 T cell response to murine cytomegalovirus. J. Immunol. **176:**3760–3766.
37. **Rawlinson, W. D., H. E. Farrell, and B. G. Barrell.** 1996. Analysis of the complete DNA sequence of murine cytomegalovirus. J. Virol. **70:**8833–8849.
38. **Razonable, R. R.** 2005. Epidemiology of cytomegalovirus disease in solid organ and hematopoietic stem cell transplant recipients. Am. J. Health Syst. Pharm. **62(8 Suppl. 1):**S7–S13.
39. **Redpath, S., A. Angulo, N. R. Gascoigne, and P. Ghazal.** 1999. Murine cytomegalovirus infection down-regulates MHC class II expression on macrophages by induction of IL-10. J. Immunol. **162:**6701–6707.
40. **Redwood, A. J., et al.** 2005. Use of a murine cytomegalovirus K181-derived bacterial artificial chromosome as a vaccine vector for immunocontraception. J. Virol. **79:**2998–3008.
41. **Shen, Z., G. Reznikoff, G. Dranoff, and K. L. Rock.** 1997. Cloned dendritic cells can present exogenous antigens on both MHC class I and class II molecules. J. Immunol. **158:**2723–2730.
42. **Smith, H. R., et al.** 2002. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. Proc. Natl. Acad. Sci. U. S. A. **99:**8826–8831.
43. **Snyder, C. M., et al.** 2009. $CD4^+$ T cell help has an epitope-dependent impact on $CD8^+$ T cell memory inflation during murine cytomegalovirus infection. J. Immunol. **183:**3932–3941.
44. **Varani, S., et al.** 2005. Impaired dendritic cell immunophenotype and function in heart transplant patients undergoing active cytomegalovirus infection. Transplantation **79:**219–227.
45. **Whitmire, J. K., et al.** 1999. CD40-CD40 ligand costimulation is required for generating antiviral CD4 T cell responses but is dispensable for CD8 T cell responses. J. Immunol. **163:**3194–3201.
46. **Wiesel, M., S. Walton, K. Richter, and A. Oxenius.** 2009. Virus-specific CD8 T cells: activation, differentiation and memory formation. APMIS **117:**356–381.
47. **Yang, Y., et al.** 1996. Transient subversion of CD40 ligand function diminishes immune responses to adenovirus vectors in mouse liver and lung tissues. J. Virol. **70:**6370–6377.
48. **Zanghellini, F., S. B. Boppana, V. C. Emery, P. D. Griffiths, and R. F. Pass.** 1999. Asymptomatic primary cytomegalovirus infection: virologic and immunologic features. J. Infect. Dis. **180:**702–707.
49. **Zhan, X., et al.** 2000. Mutagenesis of murine cytomegalovirus using a Tn3-based transposon. Virology **266:**264–274.
50. **Ziegler, H., et al.** 1997. A mouse cytomegalovirus glycoprotein retains MHC class I complexes in the ERGIC/*cis*-Golgi compartments. Immunity **6:**57–66.