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Murine Cytomegalovirus Infection Inhibits Tumor Necrosis Factor Alpha Responses in Primary Macrophages

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Despite robust host immune responses the betaherpesvirus murine cytomegalovirus (MCMV) is able to establish lifelong infection. This capacity is due at least in part to the virus utilizing multiple immune evasion mechanisms to blunt host responses. Macrophages are an important cell for MCMV infection, dissemination, and latency despite expression in the host of multiple cytokines, including tumor necrosis factor alpha (TNF-α), that can induce an antiviral state in macrophages or other cells. In this study, we found that MCMV infection of bone marrow-derived macrophages inhibited TNF-α-induced ICAM-1 surface expression and mRNA expression in infected cells via expression of immediate early and/or early viral genes. MCMV infection blocked TNF-α-induced nuclear translocation of NF-κB. This inhibition of TNF-α signaling was explained by a decrease in TNF receptor 1 (TNFR1) and TNFR2 that was due to decreased mRNA for the latter. These findings provide a mechanism by which MCMV can evade the effects of an important host cytokine in macrophages.

The betaherpesvirus human cytomegalovirus (HCMV) maintains lifelong infection in more than 50% of the U.S. population and is present at higher levels in the residents of underdeveloped countries (24). In the immunocompetent host, this chronicity and prevalence is thought to reflect the balance between multiple viral immune evasion strategies and a correspondingly complex host immune response, resulting in a lifelong immunological stalemate (25). However, inadequate immune defense in neonates, organ transplant recipients, and AIDS patients can upset this equilibrium, resulting in devastating CMV disease (references 24 and 29 and references therein). The strict host species specificity of HCMV has led to the use of murine cytomegalovirus (MCMV) infection of mice as an excellent small-animal model of HCMV disease (reference 25 and references therein).

Acute MCMV infection results in dramatic induction of several cytokines, including interleukin-12 p40, gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukin-6 (35). Generally, these cytokines are thought to be protective through direct antiviral action and/or indirectly by orchestrating the immune response. Several studies illustrate the protective effects of TNF-α pretreatment in vitro (1, 18, 43), but the effects of TNF-α in vivo are less clear. Several laboratories have used antibodies or soluble TNF receptors to deplete TNF-α in vivo, resulting in enhanced MCMV disease (12, 15, 28, 30). When TNF-α was depleted in T- and B-cell-deficient mice, we observed decreased major histocompatibility complex class II induction on infiltrating macrophages, decreased numbers of macrophages in virus-induced inflammatory infiltrates, increased splenic MCMV titers, and increased splenic pathology (15). Other groups found no effect of TNF-α treatment or depletion under their protocol regimens despite positive controls, such as blocking lipopolysaccharide responses (38, 43). One study has shown that TNF-α pretreatment is protective in vivo, in synergy with IFN-γ (18). Treatment after MCMV infection resulted in increased mortality, illustrating that TNF-α is a two-edged sword (1). Interestingly, studies with HCMV have also revealed conflicting roles for cytokines such as TNF-α and IFN-γ in controlling viral infection in some situations (10, 11, 26) but not others (41). Studies revealing that HCMV inhibits TNF-α signaling and TNF receptor 1 (TNFR1) expression may in part resolve these conflicting findings (2).

Macrophages are important in the pathogenesis of MCMV infection. While cytokine-stimulated macrophages can limit MCMV replication at the level of decreased immediate early protein production (33), macrophages can also contribute to MCMV dissemination (3, 9, 36, 42) and maintenance via latency (5, 23, 32). Studies with mutant MCMVs defective for growth in macrophages have illustrated the important role of macrophages in determining MCMV pathogenesis (7, 13). Importantly, the complete differentiation of a macrophage into its activated state relies on stimulation by cytokines such as IFN-γ or TNF-α (4, 34, 45). Since MCMV infection is known to inhibit macrophage activation by IFN-γ (14), we tested the hypothesis that MCMV can also inhibit TNF-α action in primary macrophages. We found that MCMV infection inhibits TNF-α-induced gene expression in macrophages and that this was associated with defective NF-kB translocation to the nucleus and decreased expression of TNF receptors.

Results and discussion. We hypothesized that MCMV may have evolved mechanisms to inhibit TNF-α signaling, given the importance of TNF-α in macrophage activation (34, 45) and the likely role of TNF-α in controlling MCMV infection (12, 15, 18, 28, 30). To address this hypothesis, we infected bone marrow-derived (BM) macrophages with MCMV at a multiplicity of infection (MOI) of 10 for 18 h and then stimulated

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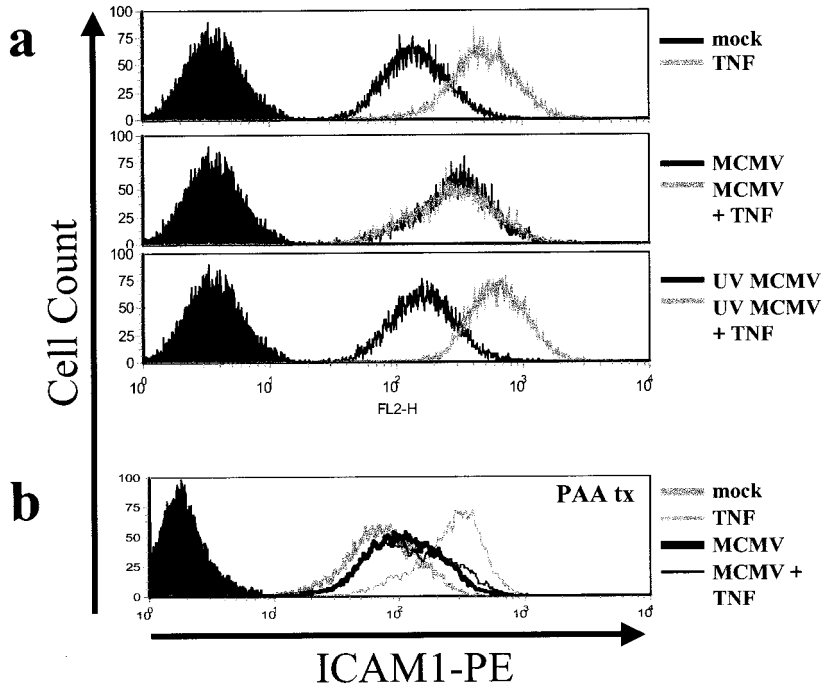

FIG. 1. MCMV IE and/or E genes block TNF-α-induced ICAM-1 expression. (a) FACS analysis of BM macrophages for surface ICAM-1 expression. (b) BM macrophages were infected and stimulated as done for panel a with the addition of phosphonoacetic acid (PAA) treatment. Isotype control is represented by filled histograms in panels a and b. Results of one representative experiment of three independent experiments are shown.

the cultures with 10 ng of TNF-α/ml for 12 h. This MOI infected >95% of the macrophages (D. L. Popkin, M. Watson, E. Karaskov, G. P. Dunn, R. Bremner, and H. W. Virgin, submitted for publication). UV-inactivated virus (14), Smith strain MCMV, or media alone were used to infect BM macrophages as described previously (14). Cells were harvested for fluorescence-activated cell sorter (FACS) analysis of the TNF-α-induced adhesion molecule ICAM-1 (17) (Fig. 1) by using the 3E2 monoclonal antibody specific for ICAM-1 (Pharmingen) (14). MCMV infection alone results in a modest up-regulation of ICAM-1 (Fig. 1a). Uninfected macrophages showed a robust up-regulation of surface ICAM-1 expression after TNF-α treatment (Fig. 1a). However, infected BM macrophages were unresponsive to TNF-α induction of surface ICAM-1 expression. MCMV inhibition of TNF-α-induced ICAM-1 expression required live virus, since UV-inactivated MCMV did not block TNF-α induction of ICAM-1 and was specific to ICAM-1, as MCMV infection does not affect the surface expression of IFNGR1 (14). Viral DNA synthesis and late gene expression were not required, since viral inhibition of TNF-α-induced ICAM-1 surface expression was not sensitive to phosphonoacetic acid at 400 μg/ml or cidofovir treatment at 2 μg/ml at the time of infection and at the time of stimulation (Fig. 1b and data not shown) (27, 37).

We next asked whether MCMV immediate early and/or early genes may be encoding or inducing a soluble mediator that inhibits TNF-α-inducible ICAM-1 expression in a manner similar to the strategy employed by the *Poxviridae* (20) or whether only MCMV-infected cells were unresponsive to TNF-α. We infected macrophages with an MCMV mutant expressing green fluorescent protein (GFP) (16) so that we could distinguish between infected and uninfected macrophages in infected cultures. We used an MOI of 3 so that only 20 to 50% of macrophages would be infected, and then we analyzed the response to 12 h of TNF-α stimulation at 10 ng/ml 18 h after mock or MCMV infection. When cultures were infected under these conditions, both uninfected and infected BM macrophages show a modest increase in ICAM-1 expression that we ascribe to a soluble mediator released by infected cells that acts on both infected and uninfected cells (Fig. 2). Importantly, only the infected GFP^+—and therefore MCMV-infected—macrophages failed to respond to TNF-α stimulation by increasing ICAM-1 expression (Fig. 2). This indicates that the inhibition of TNF-α responsiveness that we observed was due to direct effects of MCMV infection.

To determine the mechanism of MCMV inhibition of TNF-α-induced ICAM-1 surface expression, we analyzed ICAM-1 transcript induction by quantitative real-time reverse transcription-PCR (qRT-PCR) as described previously (Popkin et al., submitted) by using the primers ICAM1_F (5′-CTGGCATTG TTCTCTAATGTCT-3′) and ICAM1_R, 5′-GGAGCAAAAC AACTTCTGC-3′. MCMV infection of BM macrophages at an MOI of 10 inhibited TNF-α (10 ng/ml for 6 h)-induced increases in ICAM-1 mRNA levels but did not affect the levels of beta-actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Fig. 3a and data not shown) (Popkin et al., submitted). To address whether this effect was specific for ICAM-1 mRNA, we examined expression of the TRAF1 gene, another gene whose transcription is up-regulated by TNF-α, by using the primers TRAF1_F (5′-GAGAGCACCTCCTGAGC

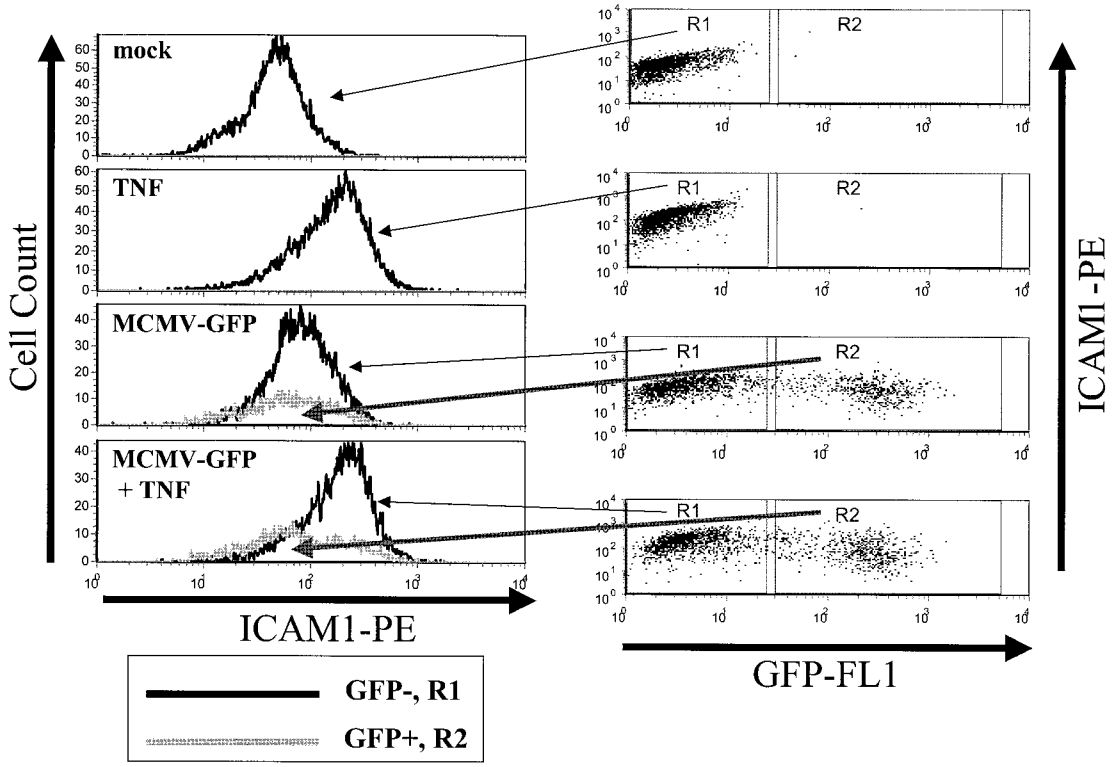

FIG. 2. Uninfected BM macrophages respond to TNF-α treatment in the presence of infected BM macrophages. Shown is FACS analysis of BM macrophages for surface ICAM-1 expression. Results of one representative experiment of six independent experiments are shown.

T-3′) and TRAF1_R (5′-GAGTCGCAGACTGTGCTC-3′). We found that TNF-α induction of TRAF1 mRNA was blocked by MCMV infection (Fig. 3a). This showed that multiple TNF-α transcriptional responses were blocked by MCMV infection (levels of beta-actin and 18S RNA were unaffected under all conditions), suggesting that MCMV might inhibit a proximal event in TNF-α signaling.

Both ICAM-1 and TRAF1 transcript induction relies on the TNF-α-induced nuclear translocation of NF-κB (8, 44). Therefore, we monitored NF-κB localization in infected cells by immunofluorescence microscopy. BM macrophages were mock or MCMV infected for 18 h and then stimulated with TNF-α (10 ng/ml) for 30 min. Macrophages were then fixed, washed, and stained for NF-κB by using the NF-κB HitKit (Cellomics) per the manufacturer's directions, yielding green color (fluorescein isothiocyanate [FITC]). Cells were counterstained with the nuclear dye bisbenzimide, yielding red color. Nuclear localization of NF-κB is indicated by yellow color. We found that TNF-α-induced nuclear translocation was absent in MCMV-infected BM macrophages (Fig. 3b). This suggested that MCMV infection might inhibit an event in TNF-α signaling proximal to activation and translocation of NF-κB in a *cis* or *trans* mechanism. Notably, MCMV is known to inhibit apoptosis (6, 21), and we subsequently tested whether MCMV could inhibit TNF-α-induced apoptosis in the presence of cycloheximide and actinomycin D. However, in BM macrophages, the levels of drug inhibitors necessary to induce apoptosis also resulted in cell death in the absence of TNF-α, as determined by annexin V staining and direct visualization. We were unable to determine if MCMV infection inhibits TNF-α-induced apoptosis in BM macrophages (data not shown).

We next determined whether MCMV might inhibit surface expression of TNF-α receptors by FACS analysis. After 18 h of MCMV or mock infection, cells were harvested and stained with hamster monoclonal antibodies to TNFR1 (p55), TNFR2 (p75), or an isotype control (Pharmingen). As shown in Fig. 4a, MCMV infection resulted in decreases in both TNFR1 and TNFR2 surface expression (mean fluorescent intensities, 26.77 and 17.81 [*P* value, 0.05] and 83.38 and 57.11 [*P* value, 0.04] for mock and MCMV infection, respectively, with comparison by two-tailed paired *t* test). This is consistent with the defects observed in TNF-α signaling (Fig. 1 to 3).

Changes in cell surface expression of TNF receptors may be due to shedding of the receptors as a mechanism of negative feedback (31). Therefore, we speculated that decreased receptor surface expression may be due to induction of TNF-α receptor shedding by MCMV infection. Soluble TNF-α, p55, and p75 were measured in cell supernatants by sandwich enzyme-linked immunosorbent assay (ELISA) as described previously (39). (Capture and detection antibodies were kindly donated by Robert D. Schreiber.) Values below background were set at the limit of detection. Quantitation of soluble TNF receptors by ELISA did not reveal increased TNF receptor shedding or secreted TNF-α due to MCMV infection (Fig. 4b and data not shown).

Therefore, we evaluated TNFR1 and TNFR2 transcript levels as a possible explanation for decreased TNF-α receptor expression in infected cells. BM macrophages were either

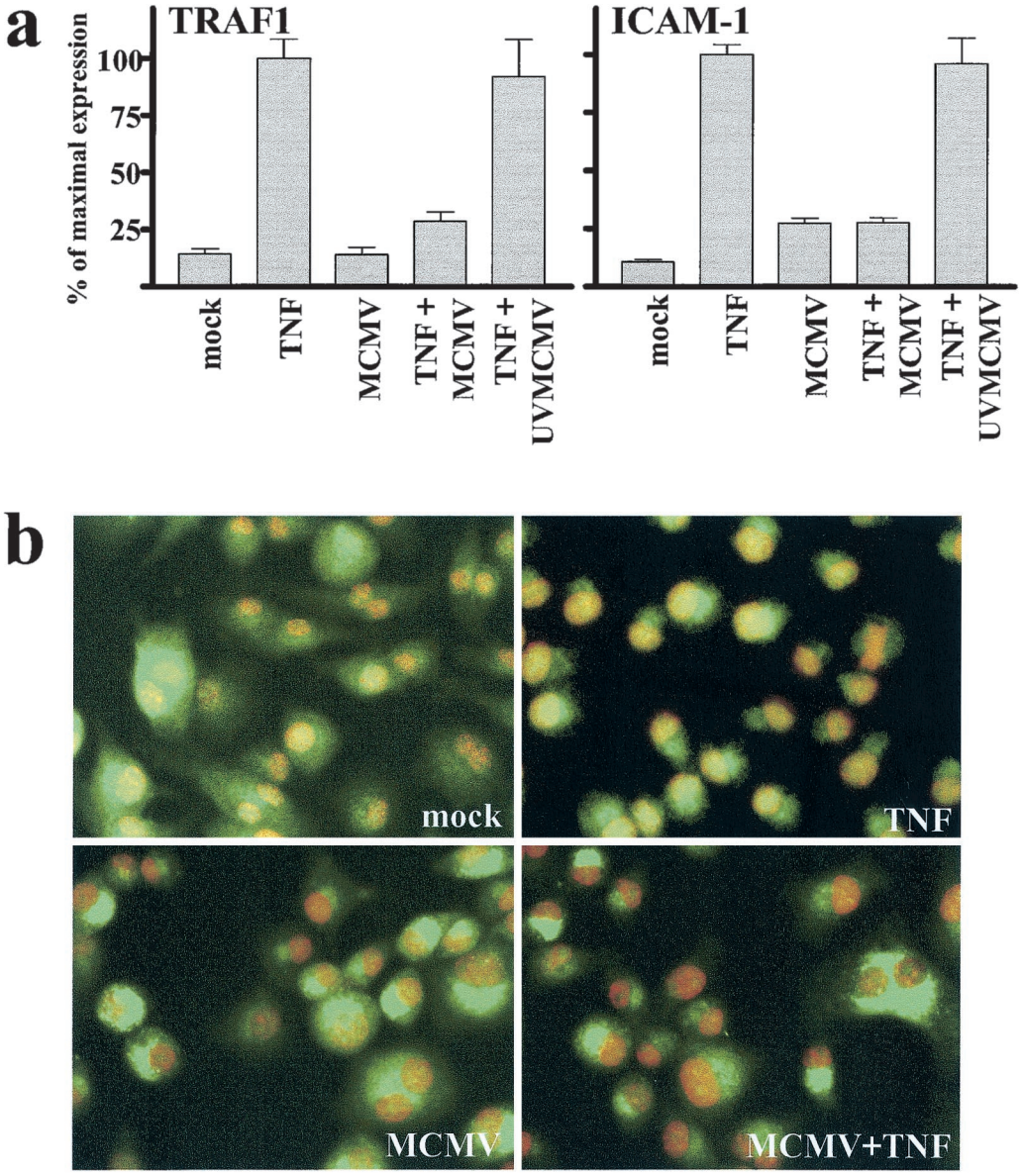

FIG. 3. MCMV inhibits TNF-α-induced transcription and NF-kB translocation in BM macrophages. (a) qRT-PCR analysis was performed to quantitate TNF-α-induced TRAF1 and ICAM-1 transcripts. The mean and standard error of the mean are shown from four independent experiments. (b) Confocal immunofluorescence studies were performed to monitor TNF-α-induced NF-κB nuclear localization in the presence or absence of MCMV infection. Results of one of three independent experiments are shown.

MCMV or mock infected, and RNA was harvested at 0, 6, 24, and 48 h postinfection for qRT-PCR analysis. The following primers were used to quantitate gene expression: p55_F, 5′-ACAGCTGCAGTTCGAAGACC-3′; p55_R, 5′-ACTTCCAGCGTGTCCTCGT-3′; p75_F, 5′-GCTGAGGCACTAGAGCTCCA-3′; p75R, 5′-AGCTGCAGTTCGAAGACCAG-3′. We found decreased levels of TNFR2, but not TNFR1, message when comparing mock versus MCMV infection of BM macrophages (Fig. 4c and d). These data are consistent with a model in which MCMV infection of BM macrophages decreased TNFR2 expression via inhibition of mRNA for the receptor and decreased TNFR1 surface expression by a posttranscriptional mechanism using a *cis* or *trans* mechanism.

In summary, we have observed a proximal blockade in TNF-α signaling in MCMV-infected primary macrophages. Several other viruses, including adenovirus, poxvirus, and African swine fever virus (19, 22, 40), have also evolved mechanisms to inhibit TNF-α signaling, supporting the idea that TNF-α is an important antiviral molecule worthy of targeting via specific viral immune evasion strategies. Interestingly, Baillie et al. have proposed that HCMV also targets TNF-α signaling in a manner similar to that reflected in the data presented in this study (2).

Importantly, our findings may help resolve a discrepancy in the literature regarding the importance of TNF-α in controlling MCMV infection. Consistent with findings by Anderson et

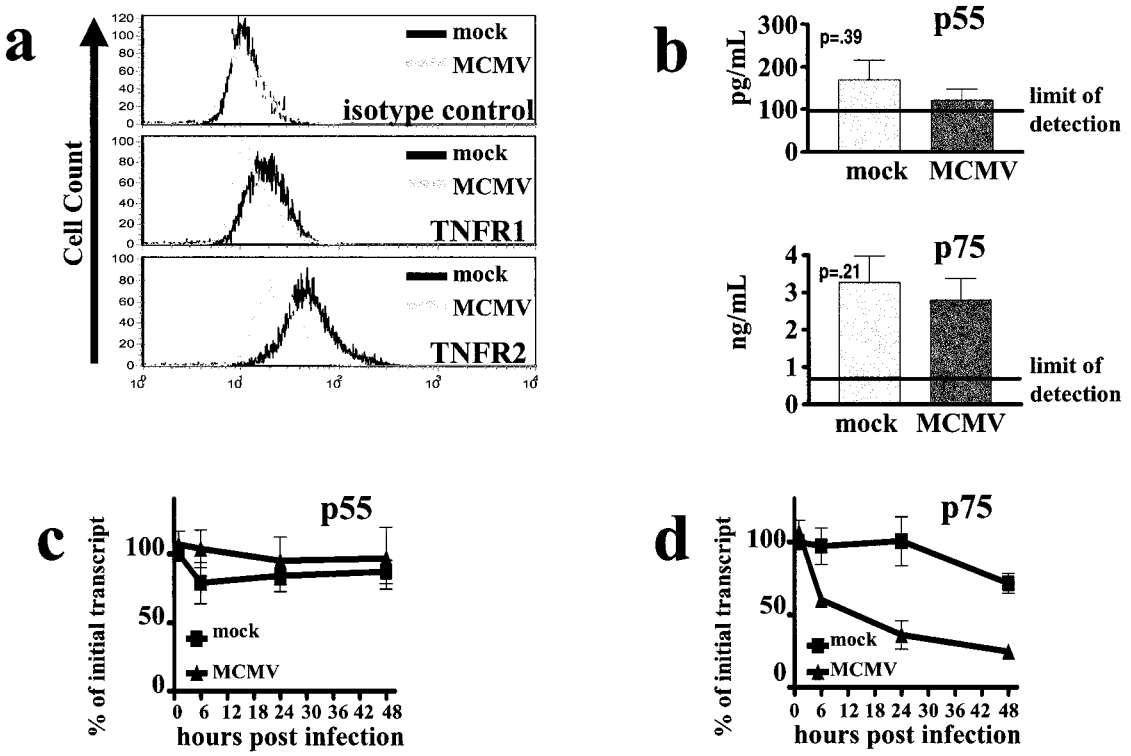

FIG. 4. MCMV decreases TNF receptor surface expression. (a) FACS analysis for TNFR1 (p55), TNFR2 (p75), or an isotype control. (b) Soluble TNF-α, p55, and p75 were measured in cell supernatants by sandwich ELISA. The means and standard errors of the mean from four independent experiments are shown along with *P* values for the Student's paired *t* test. (c and d) qRT-PCR analysis for p55 (c) and p75 (d) transcripts in BM macrophages. The means and standard errors of the mean of four independent experiments are shown.

al. (1), our data provide a mechanistic basis for the absence of a therapeutic benefit when giving TNF-α postinfection and may explain why the protective effects of TNF-α are observed only under specific experimental conditions (1, 38, 43). It is interesting that MCMV inhibits both IFN-γ and TNF-α signaling. These two cytokines potently synergize to block MCMV replication in fibroblasts, arguing that inhibiting both pathways might be important for MCMV to replicate effectively in the face of innate and adaptive immune responses.

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REFERENCES

1. **Anderson, K. P., Y. S. Lie, M. A. Low, and E. H. Fennie.** 1993. Effects of tumor necrosis factor-alpha treatment on mortality in murine cytomegalovirus-infected mice. Antivir. Res. **21:**343–355.
2. **Baillie, J., D. Sahlender, and J. H. Sinclair.** 2003. Human cytomegalovirus infection inhibits tumor necrosis factor alpha (TNF-alpha) signaling by targeting the 55-kilodalton TNF-alpha receptor. J. Virol. **77:**7007–7016.
3. **Bale, J. F., Jr., and M. E. O'Neil.** 1989. Detection of murine cytomegalovirus DNA in circulating leukocytes harvested during acute infection of mice. J. Virol. **63:**2667–2673.
4. **Billiau, A., H. Heremans, K. Vermeire, and P. Matthys.** 1998. Immunomodulatory properties of interferon-gamma. An update. Ann. N. Y. Acad. Sci. **856:**22–32.
5. **Brautigam, A. R., F. J. Dutko, L. B. Olding, and M. B. Oldstone.** 1979. Pathogenesis of murine cytomegalovirus infection: the macrophage as a permissive cell for cytomegalovirus infection, replication, and latency. J. Gen. Virol. **44:**349–359.
6. **Brune, W., C. Menard, J. Heesemann, and U. H. Koszinowski.** 2001. A ribonucleotide reductase homolog of cytomegalovirus and endothelial cell tropism. Science **291:**303–305.
7. **Cavanaugh, V. J., R. M. Stenberg, T. L. Staley, H. W. Virgin, M. R. MacDonald, S. Paetzold, H. E. Farrell, W. D. Rawlinson, and A. E. Campbell.** 1996. Murine cytomegalovirus with a deletion spanning *Hin*dIII-J and -I displays altered cell and tissue tropism. J. Virol. **70:**1365–1374.
8. **Chen, C., C. Chou, Y. Sun, and W. Huang.** 2001. Tumor necrosis factor alpha-induced activation of downstream NF-kappaB site of the promoter mediates epithelial ICAM-1 expression and monocyte adhesion. Involvement of PKCalpha, tyrosine kinase, and IKK2, but not MAPKs, pathway. Cell Signal. **13:**543–553.
9. **Collins, T. M., M. R. Quirk, and M. C. Jordan.** 1994. Biphasic viremia and viral gene expression in leukocytes during acute cytomegalovirus infection of mice. J. Virol. **68:**6305–6311.
10. **Davignon, J. L., P. Castanie, J. A. Yorke, N. Gautier, D. Clement, and C. Davrinche.** 1996. Anti-human cytomegalovirus activity of cytokines produced by $CD4^+$ T-cell clones specifically activated by IE1 peptides in vitro. J. Virol. **70:**2162–2169.
11. **Gamadia, L. E., E. B. Remmerswaal, J. F. Weel, F. Bemelman, R. A. van Lier, and I. J. ten Berge.** 2003. Primary immune responses to human CMV: a critical role for IFN-gamma-producing CD4+ T cells in protection against CMV disease. Blood **101:**2686–2692.
12. **Gariglio, M., P. Foresta, C. Sacchi, M. Lembo, L. Hertel, and S. Landolfo.** 1997. Suppression of high mobility group protein T160 expression impairs mouse cytomegalovirus replication. J. Gen. Virol. **78:**665–670.
13. **Hanson, L. K., J. S. Slater, Z. Karabekian, H. W. Virgin, C. A. Biron, M. C. Ruzek, N. van Rooijen, R. P. Ciavarra, R. M. Stenberg, and A. E. Campbell.** 1999. Replication of murine cytomegalovirus in differentiated macrophages as a determinant of viral pathogenesis. J. Virol. **73:**5970–5980.
14. **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 MHC class II associated genes. J. Exp. Med. **187:**1037–1046.
15. **Heise, M. T., and H. W. Virgin.** 1995. The T-cell-independent role of gamma interferon and tumor necrosis factor alpha in macrophage activation during murine cytomegalovirus and herpes simplex virus infections. J. Virol. **69:** 904–909.

16. **Henry, S. C., K. Schmader, T. T. Brown, S. E. Miller, D. N. Howell, G. G. Daley, and J. D. Hamilton.** 2000. Enhanced green fluorescent protein as a marker for localizing murine cytomegalovirus in acute and latent infection. J. Virol. Methods **89:**61–73.
17. **Kharbanda, S., T. Nakamura, R. Datta, M. L. Sherman, and D. Kufe.** 1990. Induction of monocytic differentiation by tumor necrosis factor in phorbol ester-resistant KG-1a cells. Cancer Commun. **2:**327–332.
18. **Lucin, P., S. Jonjic, M. Messerle, B. Polic, H. Hengel, and U. H. Koszinowski.** 1994. Late phase inhibition of murine cytomegalovirus replication by synergistic action of interferon-gamma and tumor necrosis factor. J. Gen. Virol. **75:**101–110.
19. **Mahr, J. A., and L. R. Gooding.** 1999. Immune evasion by adenoviruses. Immunol. Rev. **168:**121–130.
20. **McFadden, G., M. Schreiber, and L. Sedger.** 1997. Myxoma T2 protein as a model for poxvirus TNF receptor homologs. J. Neuroimmunol. **72:**119–126.
21. **Menard, C., M. Wagner, Z. Ruzsics, K. Holak, W. Brune, A. E. Campbell, and U. H. Koszinowski.** 2003. Role of murine cytomegalovirus US22 gene family members in replication in macrophages. J. Virol. **77:**5557–5570.
22. **Miskin, J. E., C. C. Abrams, L. C. Goatley, and L. K. Dixon.** 1998. A viral mechanism for inhibition of the cellular phosphatase calcineurin. Science **281:**562–565.
23. **Mitchell, B. M., A. Leung, and J. G. Stevens.** 1996. Murine cytomegalovirus DNA in peripheral blood of latently infected mice is detectable only in monocytes and polymorphonuclear leukocytes. Virology **223:**198–207.
24. **Mocarski, E. S., and C. T. Courcelle.** 2001. Cytomegaloviruses and their replication, p. 2629–2674. *In* D. Knipe and P. Howley (ed.), Fields virology. Lippincott Williams & Wilkins, New York, N.Y.
25. **Mocarski, E. S., Jr.** 2002. Immunomodulation by cytomegaloviruses: manipulative strategies beyond evasion. Trends Microbiol. **10:**332–339.
26. **Nordoy, I., F. Muller, K. P. Nordal, H. Rollag, E. Lien, P. Aukrust, and S. S. Froland.** 2000. The role of the tumor necrosis factor system and interleukin-10 during cytomegalovirus infection in renal transplant recipients. J. Infect. Dis. **181:**51–57.
27. **Okleberry, K. M., R. P. Warren, and D. F. Smee.** 1997. Metabolism of ganciclovir and cidofovir in cells infected with drug-resistant and wild-type strains of murine cytomegalovirus. Antivir. Res. **35:**83–90.
28. **Orange, J. S., and C. A. Biron.** 1996. Characterization of early IL-12, IFN-alpha/beta, and TNF effects on antiviral state and NK cell responses during murine cytomegalovirus infection. J. Immunol. **156:**4746–4756.
29. **Pass, R. F.** 2001. Cytomegalovirus, p. 2675–2706. *In* D. Knipe and P. Howley (ed.), Fields virology. Lippincott Williams & Wilkins, New York, N.Y.
30. **Pavic, I., B. Polic, I. Crnkovic, P. Lucin, S. Jonjic, and U. H. Koszinowski.** 1993. Participation of endogenous tumour necrosis factor-alpha in host resistance to cytomegalovirus infection. J. Gen. Virol. **74:**2215–2223.
31. **Pinckard, J. K., K. C. Sheehan, C. D. Arthur, and R. D. Schreiber.** 1997. Constitutive shedding of both p55 and p75 murine TNF receptors in vivo. J. Immunol. **158:**3869–3873.
32. **Pollock, J. L., R. M. Presti, S. Paetzold, and H. W. Virgin.** 1997. Latent murine cytomegalovirus infection in macrophages. Virology **227:**168–179.
33. **Presti, R. M., D. L. Popkin, M. Connick, S. Paetzold, and H. W. Virgin.** 2001. Novel cell type-specific antiviral mechanism of interferon gamma action in macrophages. J. Exp. Med. **193:**483–496.
34. **Riches, D. W., E. D. Chan, and B. W. Winston.** 1996. TNF-alpha-induced regulation and signalling in macrophages. Immunobiology **195:**477–490.
35. **Ruzek, M. C., A. H. Miller, S. M. Opal, B. D. Pearce, and C. A. Biron.** 1997. Characterization of early cytokine responses and an interleukin (IL)-6-dependent pathway of endogenous glucocorticoid induction during murine cytomegalovirus infection. J. Exp Med. **185:**1185–1192.
36. **Saltzman, R. L., M. R. Quirk, and M. C. Jordan.** 1988. Disseminated cytomegalovirus infection. Molecular analysis of virus and leukocyte interactions in viremia. J. Clin. Investig. **81:**75–81.
37. **Sandford, G. R., J. R. Wingard, J. W. Simons, S. P. Staal, R. Saral, and W. H. Burns.** 1985. Genetic analysis of the susceptibility of mouse cytomegalovirus to acyclovir. J. Virol. **54:**104–113.
38. **Shanley, J. D., E. Goff, R. J. Debs, and S. J. Forman.** 1994. The role of tumor necrosis factor-alpha in acute murine cytomegalovirus infection in BALB/c mice. J. Infect. Dis. **169:**1088–1091.
39. **Sheehan, K. C., J. K. Pinckard, C. D. Arthur, L. P. Dehner, D. V. Goeddel, and R. D. Schreiber.** 1995. Monoclonal antibodies specific for murine p55 and p75 tumor necrosis factor receptors: identification of a novel in vivo role for p75. J. Exp. Med. **181:**607–617.
40. **Smith, G. L., J. A. Symons, A. Khanna, A. Vanderplasschen, and A. Alcami.** 1997. Vaccinia virus immune evasion. Immunol. Rev. **159:**137–154.
41. **Soderberg-Naucler, C., K. N. Fish, and J. A. Nelson.** 1997. Interferon-gamma and tumor necrosis factor-alpha specifically induce formation of cytomegalovirus-permissive monocyte-derived macrophages that are refractory to the antiviral activity of these cytokines. J. Clin. Investig. **100:**3154–3163.
42. **Stoddart, C. A., R. D. Cardin, J. M. Boname, W. C. Manning, G. B. Abenes, and E. S. Mocarski.** 1994. Peripheral blood mononuclear phagocytes mediate dissemination of murine cytomegalovirus. J. Virol. **68:**6243–6253.
43. **van der Meer, J. W., R. H. Rubin, M. Pasternack, D. N. Medearis, P. Lynch, and C. A. Dinarello.** 1989. The in vivo and in vitro effects of interleukin-1 and tumor necrosis factor on murine cytomegalovirus infection. Biotherapy **1:**227–231.
44. **Wang, C. Y., M. W. Mayo, R. G. Korneluk, D. V. Goeddel, and A. S. Baldwin, Jr.** 1998. NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science **281:**1680–1683.
45. **Winston, B. W., P. M. Krein, C. Mowat, and Y. Huang.** 1999. Cytokine-induced macrophage differentiation: a tale of 2 genes. Clin. Invest. Med. **22:**236–255.