Differential effects on nitric oxide-mediated vasodilation in mesenteric and uterine arteries from cytomegalovirus-infected mice

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Gombos RB, Hemmings DG. Differential effects on nitric oxide-mediated vasodilation in mesenteric and uterine arteries from cytomegalovirus-infected mice. Am J Physiol Heart Circ Physiol 299: H1124–H1134, 2010.—Chronic cytomegalovirus (CMV) infections are implicated in vascular diseases. Recently, we showed that an active mouse CMV (mCMV) infection in nonpregnant mice increased endothelial-dependent vasodilation in isolated mesenteric and uterine arteries. In late pregnancy, while increased vasodilation was found in mesenteric arteries from infected mice, there was a dramatic decrease in uterine arteries. Understanding the mechanisms for these vascular changes during CMV infections is important for pregnancy outcomes and long-term consequences of this chronic infection. Increased nitric oxide (NO) is implicated in CMV-associated atherosclerosis, and CMV replication is dependent on prostaglandin H synthase (PGHS) activity. Alternatively, CMV infections decrease NO under inflammatory conditions. We therefore hypothesized that changes in the contribution by NO or PGHS-induced vasodilators would explain the increased or decreased endothelial-dependent vasodilation in arteries from nonpregnant and late pregnant mice, respectively. We found that the contribution by NO to methacholine-induced vasodilation was significantly increased in mesenteric, but not uterine, arteries isolated from nonpregnant and pregnant mCMV-infected mice. Prostaglandin inhibition did not affect endothelial-dependent vasodilation in any group. Vasodilation responses to sodium nitroprusside, an NO donor, were increased in mesenteric and uterine arteries isolated only from mCMV-infected nonpregnant mice. These results explain the increased vasodilation responses observed in mesenteric arteries from mCMV-infected mice; however, the decreased vasodilation in uterine arteries from pregnant mice could not be explained by these mechanisms. Thus CMV infection affects the contribution of NO differently in endothelial-dependent vasodilation as a result of mCMV infection in pregnant and nonpregnant females. In contrast, there was a dramatic decrease in endothelial-dependent vasodilation along with increased vasoconstriction responses in the uterine arteries from infected compared with uninfected mice. Thus an active mCMV infection differentially affects vascular responses in these two vascular beds and also in pregnant compared with nonpregnant mice. However, the dilatory pathways affected in infected mice through which these differential effects occur are unknown and are the focus of the present study.

The balance of vasoactive factors contributing to vascular tone can vary greatly among or even within vascular beds (18). In pregnancy, the uterine artery has greater capacity for vasodilation than the mesenteric artery (37). In addition, the response to some vasoconstrictors like angiotensin II is decreased in uterine arteries during pregnancy, whereas sensitivity to other vascular agonists such as phenylephrine (PE) is increased (8, 28). In contrast, systemic arteries such as those in the mesentery do not increase in diameter to the same extent as uterine arteries during pregnancy and respond differently to some agonists, such as reduced sensitivity to PE (8). These vascular bed-dependent differences ensure that sufficient blood flow reaches the placenta and fetus during gestation while simultaneously maintaining maternal blood pressure (37).

A CMV infection affects the expression and activity of vasoactive factors such as NO and prostaglandins; however, to date, these effects have only been shown in nonvascular tissues or in isolated endothelial cell cultures. An infection with CMV infections a wide range of cell types including epithelial, endothelial, and smooth muscle cells (3). A fully productive HCMV infection (active) or viral attachment alone stimulates an inflammatory response involving prostaglandins, reactive oxygen species, and cytokines (6, 44, 54). Virus-induced production of inflammatory cytokines (24) and increased expression of adhesion molecules on the surface of infected endothelial cells (39) implicates HCMV in endothelial dysfunction and consequential vascular inflammatory diseases such as atherosclerosis and coronary heart disease (11, 14). However, little is known about the effects of CMV infection on vascular responses under otherwise normal conditions or in mildly inflammatory or stressful conditions such as pregnancy.

 Recently, we set up a mouse model to study the vascular effects of CMV infection in pregnant and nonpregnant females. We found increased endothelial-dependent vasodilation in mesenteric and uterine arteries isolated from nonpregnant mice with an active mouse CMV (mCMV) infection. In late pregnancy, we continued to see increased endothelial-dependent vasodilation in mesenteric arteries from infected compared with uninfected mice. In contrast, there was a dramatic decrease in endothelial-dependent vasodilation along with increased vasoconstriction responses in the uterine arteries from infected compared with uninfected late pregnant mice (13). Thus an active mCMV infection differentially affects vascular responses in these two vascular beds and also in pregnant compared with nonpregnant mice. However, the dilatory pathways affected in infected mice through which these differential effects occur are unknown and are the focus of the present study.

The balance of vasoactive factors contributing to vascular tone can vary greatly among or even within vascular beds (18). In pregnancy, the uterine artery has greater capacity for vasodilation through increased nitric oxide (NO) and prostacyclin (2) and also undergoes dramatic remodeling including hypertrophy and hypotrophy (37). In addition, the response to some vasoconstrictors like angiotensin II is decreased in uterine arteries during pregnancy, whereas sensitivity to other vascular agonists such as phenylephrine (PE) is increased (8, 28). In contrast, systemic arteries such as those in the mesentery do not increase in diameter to the same extent as uterine arteries during pregnancy (37) and respond differently to some agonists, such as reduced sensitivity to PE (8). These vascular bed-dependent differences ensure that sufficient blood flow reaches the placenta and fetus during gestation while simultaneously maintaining maternal blood pressure (37).

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can either increase (48) or decrease (41) nitric oxide synthase (NOS) activity, thereby affecting the levels of NO. In addition, prostaglandin H synthase (PGHS)-2 expression is increased during CMV infection to facilitate viral replication in fibroblasts (55) and retinal epithelial cells (20). It is not yet known whether PGHS-2 activity is similarly affected in vascular cells. This is important since an increase in PGHS-2 activity can affect vascular tone through increased production of the vasodilator prostacyclin and/or the vasoconstrictor thromboxane (9). However, the effects of an active CMV infection in vivo on the contribution of NO or prostaglandins to vasodilation responses have not been studied in isolated intact blood vessels.

To understand how an active CMV infection contributes to abnormal vascular responses in our mouse model and to cardiovascular disease in general, it is important to investigate the effects of infection on the vasoactive mediators that regulate vascular tone. We hypothesize that the increased endothelial-dependent vasodilation observed in mesenteric and uterine arteries from nonpregnant (NP) mice and mesenteric arteries from late pregnant (LP) mice is mediated by an increased contribution by NO and/or an increase in the balance of vasoconstrictor to vasodilator prostaglandins. We further hypothesize that the decreased endothelial-dependent vasodilation in uterine arteries of LP mice is mediated by decreased NO contribution and/or an increase in the balance of vasoconstrictor to vasodilator prostaglandins.

METHODS

Animal and tissue preparation. Virgin C57BL/6J female mice (2–4 mo of age) purchased from Jackson Laboratories were housed in Health Sciences Laboratory Animal Services at the University of Alberta. RM427+, a mCMV containing a lacZ insertion in the nonessential immediate-early 2 gene (gift from E. Mocarski, Stanford University, Stanford, CA) was propagated in mouse fibroblasts (45). NP mice were given 106 plaque-forming units (PFU) of RM427+ by intraperitoneal injection 1 wk before breeding (13), a dose that will induce an effective immune response but not produce visible harmful effects (34). Pregnant mice were euthanized on day 18 of a 19-day gestation (LP). All animals were euthanized by cervical dislocation within 1 mo of infection (5–14 days for NP; 23–31 days for LP). This corresponds to an active infection (51) with detectable infectious virus in all tissues tested from both groups of infected mice (13). The uterine horns and mesentery were isolated from each mouse and immediately placed in ice-cold N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid-buffered physiological saline solution (HEPES-PSS; in mM: 10 HEPES, 1.56 CaCl2, 142 NaCl, 4.7 KCl, 1.18 KH2PO4, 1.17 MgSO4, and 5.5 glucose at pH 7.5). These animal protocols mounted and tied onto a glass cannula (80–100–6 for exact number of animals used). One end of each artery was taken from a minimum of four animals per treatment group (see Figs. 1–6 for exact number of animals used). The other end of each artery with a low flow (<10 μl/min) of HEPES-PSS. The opposite end of the vessel was then mounted and tied onto a second cannula. The cannulated vessels were held in a 2.5-ml bath containing HEPES-PSS at 37°C that was replaced every 10 min before and after the dose-response curves. The vessels were pressurized to 60 mmHg (mesenteric arteries) or 50 mmHg (uterine arteries) for 30 min. These pressures are optimum for vascular responses in these arteries (16, 36, 50). Arteries unable to maintain pressure were discarded and replaced with new vessels from the same animals. For each artery type (mesenteric and uterine), two vessels from the same vascular bed were mounted at the same time in the dual-chamber arteriograph. The arteries were equilibrated for 10 min each at 20 mmHg, at 40 mmHg, and at the final pressure, with length adjustments to remove kinks in the vessel. After equilibration, initial lumen diameter was measured via a charge-coupled device (CCD) video camera module (Sony) connected to a compound microscope. Lumen diameter was measured digitally with a video dimension analyzer.

Experimental design. For each artery type, one vessel was treated with the neuronal (NOS1 or nNOS)/inducible (NOS2 or iNOS)/endothelial (NOS3 or eNOS) NOS inhibitor Nω-nitro-l-arginine methyl ester (l-NAME; Calbiochem) at 100 μM, the PGHS-1/2 inhibitor meclofenamate (Sigma) at 1 μM, the thromboxane receptor antagonist SQ-29548 (Cayman Chemical) at 10 μM, or a combination of l-NAME with meclofenamate or l-NAME with SQ-29548. For each inhibitor tested, a second untreated vessel was always tested simultaneously to provide a direct comparison of vessels taken from the same animal. After measurement of the initial lumen diameter, one or both inhibitors were incubated for 30 min in one of the two baths while the other artery remained untreated. We did not observe any differences in baseline vascular tone in response to the addition of any inhibitor or combination of inhibitors. After the lumen diameters were recorded, both arteries were then preconstricted 40–60% with 0.5 μM U-46619 (Calbiochem), a thromboxane mimetic (mesentery), or 0.3 μM PE (Sigma), an α1-adrenergic agonist (uterine). There were no significant differences in the agonist concentrations needed to achieve 40–60% constriction in the presence or absence of any inhibitors in NP or LP mice. In our previous study (13), we found that mesenteric arteries from pregnant mice did not maintain constriction to PE over the time required to complete a vasodilation experiment. Therefore, to be consistent with the previous study, we used U-46619 in mesenteric arteries and PE in uterine arteries for preconstriction. Endothelial-dependent vasodilation was then assessed in response to increasing concentrations of methacholine (ME; 1 nM to 10 μM), or 0.3 μM PE (Sigma), an α1-adrenergic agonist (uterine). There were no significant differences in the agonist concentrations needed to achieve 40–60% constriction in the presence or absence of any inhibitors in NP or LP mice. In our previous study (13), we found that mesenteric arteries from pregnant mice did not maintain constriction to PE over the time required to complete a vasodilation experiment. Therefore, to be consistent with the previous study, we used U-46619 in mesenteric arteries and PE in uterine arteries for preconstriction. Endothelial-dependent vasodilation was then assessed in response to increasing concentrations of methacholine (ME; 1 nM to 10 μM, Sigma). ME-induced vasodilation in uterine arteries preconstricted with U-46619 showed results consistent with those found with PE in a subset of animals (data not shown). Endothelial-independent vasodilation was assessed in response to sodium nitroprusside (SNP; 0.1 nM to 10 μM, Sigma). Percent dilation was calculated as L2/L1 × 100, where L1 is the initial lumen diameter and L2 is the lumen diameter after vasodilator addition. The percent dilation was then normalized to the vessel diameter when fully relaxed at the same pressure (passive lumen diameter). Passive lumen diameter was recorded after a thorough washout with Ca2+-free EGTA PSS (in mM: 10 HEPES, 142 NaCl, 4.7 KCl, 1.18 KH2PO4, 1.17 MgSO4, and 5.5 glucose at pH 7.5). These animal protocols followed the guidelines outlined by the Canada Council of Animal Care and were approved by the University of Alberta Animal Welfare Committee.

Myograph studies. The second-order mesenteric and main uterine arteries (each between 150 and 300 μm) were dissected free of adipose and connective tissues in HEPES-PSS. Each artery type was followed the guidelines outlined by the Canada Council of Animal Care and were approved by the University of Alberta Animal Welfare Committee.

Quantitative RT-PCR. Quantitative RT-PCR (RT-qPCR) was performed on dissected arteries immediately snap frozen after dissection and stored at −80°C. For each reaction, arteries were pooled from 5–10 animals of the same status, and RNA was extracted with TRIzol reagent. RNA was diluted in 10 μl of RNase-free sterile water, and concentration was determined with the Nanodrop (ND-1000 Spectrophotometer; Nanodrop Technologies, Wilmington, DE) to ensure it was ≥200 ng/μl. RT was performed with the qScript cDNA supermix

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Quantas Biosciences, Gaithersburg, MD) and 200 ng/μl of extracted RNA. One-tenth (20 ng/μl) of the cDNA samples were used for real-time PCR within 24 h of the RT reaction. Real-time PCR was run with the Perfecta qPCR Fastmix (Quantas Biosciences), 20 ng/μl of cDNA obtained from the RT reaction, and NOS3-specific (12) and β-actin (27) specific primers and probes (20-μl reaction). The forward NOS3 primer (5’ to 3’) was TCTGCGCGATGTCACTATG, the reverse NOS3 primer (5’ to 3’) was CATGCGGCCTTCGTGGT, and the NOS3 probe (5’ to 3’) was CACCGCTTCGAAACCGTGC. The β-actin probe and primers were predesigned for TaqMan assays from Applied Biosystems (no. 4352933E). The PCR conditions were 1 cycle at 45°C for 2 min, 1 cycle at 95°C for 30 s, and then 40 cycles of the amplification step [95°C for 10 s and 60°C (β-actin) or 58°C (NOS3) for 30 s] in the iCycler. To determine the amount of NOS3 mRNA in each sample, levels were normalized to the positive control β-actin as an mRNA ratio (38). PCR was repeated three or four times, and samples that did not produce reliable β-actin results (cycle threshold > 29 cycles) were not included in the analysis.

**Immunofluorescence.** Tissues containing mesenteric and uterine arteries from three infected and three uninfected pregnant mice were fixed in 4% formaldehyde overnight and further incubated in 30% sucrose overnight. Tissues were then embedded into Tissue-Tek OCT embedding medium (VWR), snap-frozen in liquid nitrogen, and stored at −80°C. The frozen tissues were sliced on a cryostat in 5-μm sections, mounted onto glass slides, dried overnight, and stored at −80°C. Before staining, slides were thawed, dried for 1 h, and fixed in 100% methanol for 10 min at −20°C. Slides were immediately rinsed with three 10-min washes of PBS, and sections were then separated with a PAP pen. Each section was blocked with 10% normal goat serum (Cedarlane) in PBS for 1 h. After removal of the blocking agent, primary antibody or blocking agent (antibody diluent; negative control) was applied to each section and incubated at 4°C overnight. The primary antibodies used were rabbit polyclonal NOS2 (2 μg/ml; Abcam) and rabbit polyclonal NOS1 (1:800 dilution of whole serum; Abcam). After three 5-min washes with PBS, each section was incubated with Alexa Fluor 488 goat anti-rabbit (10 μg/ml secondary antibody (Molecular Probes) for 45 min in the dark. After three 5-min washes with PBS, 4’,6-diamidino-2-phenylindole (DAPI, 0.915 mg/ml; Invitrogen) was added for 15 min in the dark to stain the nuclei. Slides were then washed again with three 5-min washes of PBS, and 45 μl of Vectashield H:1000 (Vector Laboratories, Burlington, CA) was applied to each section. Each slide was sealed with a coverslip and stored in the dark at 4°C. Stained sections were viewed with an Olympus IX81 fluorescent microscope (Olympus), and images were captured with Slidebook 2D, 3D TimeLapse Imaging Software (Intelligent Imaging Innovations).

**Statistics.** Myography results were averaged by group (uninfected and infected, NP and LP, mesentery and uterine) and treatment. Results that fell at least 2 SD from the mean were not included in the analysis (<5%, which did not differ between animal groups). All vascular results were compared by repeated-measures two-way ANOVA followed by Holm-Sidak post hoc analysis to determine significance (*P* < 0.05). The mean area under each dose-response curve was calculated. The mean area under the inhibitor curve was then subtracted from the mean area under the control curve for each data set. The single value generated for the vessels from uninfected mice was then compared with that generated for infected mice with a Student’s t-test (*P* < 0.05). mRNA ratios from RT-qPCR were compared with a one-way ANOVA with a Bonferroni post hoc analysis to determine significance (*P* < 0.05).

**RESULTS**

**Contribution of NO and prostaglandins to endothelial-dependent vasodilation responses in mesenteric arteries.** In agreement with our previous findings (13), there was increased endothelial-dependent vasodilation in mesenteric arteries from mCMV-infected compared with uninfected NP mice (Fig. 1). We have now found that the contribution of NO to ME-induced vasodilation, as shown by NOS inhibition with L-NAME, was significantly greater (*P* < 0.05) in mesenteric arteries from mCMV-infected compared with uninfected NP mice (Fig. 1, A and B). This is also demonstrated in Fig. 1C, where the difference between the two curves in Fig. 1B is significantly greater than the difference found in Fig. 1A. Meclofenamate (Fig. 1, D–F) treatment alone had no effect on ME-induced vasodilation. Combined pretreatment with L-NAME and meclofenamate in mesenteric arteries from uninfected NP mice inhibited dilation similarly to that seen with L-NAME treatment alone (Fig. 1, A and G). In mesenteric arteries from mCMV-infected NP mice, this combined pretreatment also inhibited ME-induced vasodilation (Fig. 1H), which did not significantly differ from uninfected NP mice (Fig. 1I). Interestingly, however, the inhibition of endothelial-dependent vasodilation in the combined presence of L-NAME and meclofenamate in mesenteric arteries from mCMV-infected NP mice (Fig. 1H) was significantly less (*P* < 0.001) than that seen with treatment with L-NAME alone in these arteries (Fig. 1B). Thus the remaining endothelial-dependent vasodilation due to endothelium-derived hyperpolarizing factors (EDHF) in arteries from infected NP mice was significantly increased compared with uninfected NP mice (Fig. 1, G and H). To examine the possibility that the vasoconstrictor thromboxane was contributing to vascular responses in the infected mice, these results were repeated with SQ-29548, a specific thromboxane receptor inhibitor, in the presence and absence of L-NAME. SQ-29548 treatment alone had no effect on ME-induced vasodilation, whereas the combination of SQ-29548 and L-NAME mimicked the results found with the combined meclofenamate and L-NAME treatment (data not shown).

In LP mice, treatment of mesenteric arteries with L-NAME significantly inhibited ME-induced vasodilation only in mCMV-infected mice, although comparison of differences in area under curve between uninfected and mCMV-infected mice did not reach significance (Fig. 2, A–C). Similar to NP mice, meclofenamate (Fig. 2, D–F) or SQ-29548 (data not shown) treatment alone had no effect in either LP group. Maximal ME-induced vasodilation was significantly decreased (*P* < 0.05) in the presence of both L-NAME and meclofenamate in arteries from mCMV-infected LP mice (Fig. 2, G–I). Similar results were found with the combination of L-NAME and SQ-29548 (data not shown). However, there were no significant differences in the contribution of EDHF to endothelial-dependent vasodilation (Fig. 2, G and H).

**Contribution of NO and prostaglandins to endothelial-dependent vasodilation responses in uterine arteries.** Overall, there was increased vasodilation in uterine arteries from mCMV-infected NP mice and decreased vasodilation in uterine arteries from infected LP mice compared with uninfected mice (data not shown), as previously published (13). ME-induced vasodilation in uterine arteries was significantly inhibited to a similar extent by L-NAME treatment in all groups (Fig. 3, A–C; Fig. 4, A–C). Similar to mesenteric arteries, meclofenamate (Fig. 3, D–F; Fig. 4, D–F) or SQ-29548 (data not shown) treatment alone had no significant effect on ME-induced vasodilation in uterine arteries from any group. In contrast to results found in mesenteric arteries, treatment with the combi-
nation of L-NAME and meclofenamate or L-NAME and SQ-29548 in uterine arteries inhibited ME-induced vasodilation in all groups to the same extent as that found with L-NAME treatment alone (Fig. 3, G–I; Fig. 4, G–I). Results for each curve were summarized and expressed as mean ± SE % increase in lumen diameter compared with the initial preconstricted diameter and normalized to the passive lumen diameter. A significant difference between points on the curves was calculated by a repeated-measures 2-way ANOVA with Holm-Sidak post hoc test. For each graph, the area under the curve was calculated and the difference between 2 curves on the same graph obtained (C, F, I). These differences in area under the curve were compared between infected and uninfected groups with a Student’s t-test. *Significant differences (P < 0.05).

Distensibility of mesenteric and uterine arteries. The distensibility of mesenteric arteries from mCMV-infected NP mice was not significantly different from that in uninfected NP mice (Fig. 6A); however, it was significantly greater in mCMV-infected compared with uninfected LP mice (P < 0.05; Fig. 6B). In uterine arteries, distensibility of arteries from both infected NP and LP mice was significantly greater than in uninfected NP and LP mice, respectively (P < 0.05; Fig. 6, C and D).
Expression of NOS enzymes in mesenteric and uterine arteries. Our functional studies were supported by evaluation of NOS enzyme expression by immunofluorescence and RT-qPCR. Given the limited quantity of vascular tissue available to study, we chose to quantitatively measure expression of the major endothelial NOS enzyme, NOS3 (eNOS), by RT-qPCR (Fig. 7). We then qualitatively assessed NOS1 and NOS2 expression by immunofluorescence (Fig. 8); the specificity of the NOS3 antibodies tested on formalin-fixed tissue even after antigen retrieval was poor, and so these were not used. NOS3 mRNA was not significantly different in uterine arteries from mCMV-infected and uninfected NP and LP mice. In contrast, in mesenteric arteries, NOS3 mRNA was significantly increased in mCMV-infected LP mice (**P < 0.005) and there was a trend toward an increase in NP mice compared with the uninfected mice (Fig. 7). The expression of NOS1 and NOS2 was increased in the uterine arteries from both mCMV-infected and uninfected LP mice compared with NP mice. An increase in expression of NOS1 was also observed when uterine arteries from mCMV-infected and uninfected NP mice were compared (Fig. 8). In mesenteric arteries, only NOS2 expression was increased in mCMV-infected compared with uninfected NP mice (data not shown).

DISCUSSION

This study is the first to show that an active CMV infection leads to an increased contribution by NO to vasodilation and that this increase is found only in mesenteric and not uterine arteries from both NP and LP mice. Our previous findings of increased vasodilation in mesenteric arteries from mCMV-infected NP mice (13) can now be explained by an increased contribution by NO, increased NOS2 and NOS3 expression, and an increased vascular smooth muscle sensitivity to NO. In
contrast, the increased vasodilation previously observed in uterine arteries from mCMV-infected NP mice (13) is likely in response to an increase in vascular smooth muscle sensitivity to NO and increased NOS1 expression in the arterial wall, particularly the vascular smooth muscle. The situation in mCMV-infected LP mice differs from that of infected NP mice and again depends on the type of artery. In late pregnancy, an increase in NOS3 mRNA expression and an increase in the contribution of NO to vasodilation in mesenteric arteries from mCMV-infected mice was observed. In uterine arteries from mCMV-infected LP mice, neither the contribution of NO to vasodilation nor the vascular smooth muscle sensitivity to NO was changed, although distensibility was greatly increased. NOS1, NOS2, and NOS3 expression in uterine arteries was also not different from that in uninfected LP mice. Thus the dramatic decrease in vasodilation previously observed in these arteries (13) remains unexplained and may be attributed to reduced EDHF. Taken together, these results provide novel evidence for differential effects during an active mCMV infection on the NO pathway that contributes to regulation of vascular tone.

Interestingly, in contrast to our study in isolated intact arteries showing an increased contribution of NO to vasodilation and increased NOS3 mRNA expression, the majority of other studies that measure NOS3 expression in cultured endothelial cells show that a CMV infection leads to decreased NOS3 expression and activity (4, 41). In addition, in the present study the increased contribution by NO to vasodilation along with increased sensitivity of the vascular smooth muscle to NO and increased distensibility in the systemic vasculature...
are indicative of hypotension rather than hypertension; however, a recent study by Cheng et al. (5) finds that an active CMV infection in young male and female mice increases arterial blood pressure and exacerbates atherosclerotic plaque formation in mice fed a high-cholesterol diet. Although we previously found reduced vasoconstriction to PE in mesenteric arteries from mCMV-infected mice, Cheng et al. (5) report increases in plasma and tissue expression of another vasoconstrictor, angiotensin II. Thus it is possible that the responses observed in our study may be part of an adaptive response to counteract overall increased vasoconstriction in the mCMV-infected mice. These discrepancies strongly support the need to study responses in the vasculature at different levels of complexity including at the cellular level, in intact ex vivo arteries, and in whole animals to fully evaluate the physiological vascular response during CMV infections.

Although NOS3 is considered an important source of NO in the vasculature, both NOS1 and NOS2 also contribute to the production of NO. Most studies investigating the relationship between CMV infections and NOS regulation in vivo have focused on the inflammation-induced NOS2 enzyme. NO production in response to CMV infection is part of an important innate immune response, and NOS2 knockout mice exhibit increased viral replication and mortality (33). NOS2 expression and activity are increased in mCMV infections, and the resultant increase in NO production in the lungs and the brain contributes to diseases such as pneumonitis (48) and developmental brain disorders (25), respectively. Increased NOS2

Fig. 4. Inhibition of ME-induced vasodilation in uterine arteries from uninfected and mCMV-infected LP mice. ME-induced vasodilation was measured in mesenteric arteries from uninfected (A, D, G) and mCMV-infected (B, E, H) LP mice after preconstriction of mesenteric arteries with PE in the presence and absence of L-NAME (A–C), meclo (D–F), or L-NAME + meclo (G–I). For each graph, the area under the curve was calculated and the difference between 2 curves on the same graph obtained (C, F, I). Results were summarized and significant differences assessed as for Fig. 1. *Significant differences ($P < 0.05$). n, No. of animals.
expression and NO are also detected in aqueous humor samples of acquired immunodeficiency syndrome (AIDS) patients with CMV retinitis (21). It is therefore likely that CMV infection in endothelial and vascular smooth muscle cells could lead to increased NO through increased expression and activity of the NOS2 enzyme. Indeed, we found increased expression of NOS2 in mesenteric arteries from both nonpregnant and pregnant mCMV-infected mice. There is no information about the effect of CMV infection on NOS1 expression in the vasculature; we have now shown that NOS1 is increased in uterine arteries from NP but not LP mCMV-infected mice. Importantly, in conditions of oxidative stress, i.e., infection and to some extent pregnancy, excess NO may combine with superoxide to produce peroxynitrite, a potent and damaging proxi-
The increased NO can also play a role in mCMV infection-induced development of atherosclerosis (5, 19, 26). Different prostaglandins stimulate either vasodilation or vasoconstriction and are produced by the PGHS enzymes. In uninfected mice, inhibition of PGHS alone in either mesenteric or uterine arteries did not affect ME-induced vasodilation in our study, demonstrating that prostaglandins were not involved in endothelial-dependent vasodilation in these vascular beds (32). Surprisingly, although CMV increases PGHS-2 expression and activity in fibroblasts and epithelial cells, where one of its products, prostaglandin E2, is important for viral replication (20, 55), we found that there was still no contribution by prostaglandins to endothelial-dependent vasodilation during a mCMV infection. However, when NOS and PGHS or NOS and the thromboxane receptor were simultaneously inhibited in mesenteric arteries from mCMV-infected NP and LP mice, the residual vasodilation due to EDHF was greater than that found with NOS inhibition alone. This suggests that during a CMV infection PGHS-mediated production of a vasoconstrictor such as thromboxane was increased. Interestingly, NO has been shown to inhibit prostacyclin production by PGHS-2 (35), an effect that could occur in our model since we found increased expression of NOS enzymes. Given that there is a balance between production of prostacyclin and thromboxane, a reduction in prostacyclin production could shift the balance toward an increased production of thromboxane (9). Inhibition of the thromboxane receptor specifically led to increased vasodilation; however, when NOS remained active, it compensated for the vasoconstriction. When both pathways were inhibited and the NO pathway could no longer mask the effects of PGHS inhibition, we observed increased vasodilation, particularly in mCMV-infected NP mice. These PGHS-mediated effects were not seen in uterine arteries.

The increased sensitivity to NO in the vascular smooth muscle in mesenteric and uterine arteries from NP mice could be caused by an increased number of smooth muscle cells since CMV is known to increase smooth muscle cell proliferation (52) and migration (30). The increased sensitivity to NO in vascular smooth muscle in either type of artery from infected NP mice was lost in infected LP mice, demonstrating differences in the effects of viral infection in the NP and LP conditions. Interestingly, the increased sensitivity to NO found in uterine arteries from infected NP mice mirrored the increased sensitivity in uninfected LP compared with NP mice. Both mesenteric and uterine artery diameters increase in pregnancy (13, 37), reflecting pregnancy-induced remodeling and increased vascular smooth muscle content (23, 49). This supports the hypothesis that increased numbers of vascular smooth muscle cells could explain increased sensitivity to NO. Another explanation for the infection-induced increase in vascular smooth muscle sensitivity to NO could be an increase in guanosine 3′,5′-cyclic monophosphate (cGMP) or soluble guanylate cyclase protein expression and activity, which normally increase in pregnancy (7, 22). More specifically, a recent study provides evidence for increased cGMP in uterine vascular smooth muscle in ovine pregnancy (40), and infection with CMV also increases cellular cGMP (1).

The results of this study demonstrate novel ways in which a CMV infection affects the vasculature. CMV infection has previously been shown to impair cellular responses in single-cultured endothelial cells (39); however, the use of functional and molecular assays in isolated intact arteries from acute mCMV-infected mice to examine changes in vasoactive mediators and signaling pathways important to regulation of...
vascular responses is novel. Importantly, these results show
dysregulation of the NO pathway and suggest the importance
for evaluation of the long-term effects of CMV infections. The
differential effects of long-term infections on the vasoactive
pathways that regulate vascular function in various vascular
beds need to be further investigated since it is clear that
long-term CMV infections in humans are associated with
endothelial dysfunction, impaired NO responses, and hyper-
tensive vascular diseases (15, 42, 46). Chronic increases in NO
in response to CMV infection have important long-term im-
plications for conditions of increased oxidative stress such as
atherosclerosis where NO is converted to damaging reactive
oxygen species such as peroxynitrite. Defining the mechanisms
by which CMV infection modulates vascular responses in
different arteries could identify ways to treat vascular compli-
cations associated with a CMV infection.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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