Impaired vascular function in mice with an active cytomegalovirus infection

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Submitted 24 September 2008; accepted in final form 28 January 2009

Gombos RB, Wolan V, McDonald K, Hemmings DG. Impaired vascular function in mice with an active cytomegalovirus infection. Am J Physiol Heart Circ Physiol 296: H937–H945, 2009. First published January 30, 2009; doi:10.1152/ajpheart.01027.2008.—Human cytomegalovirus (CMV) is implicated in vascular complications through endothelial dysfunction. However, the effect of in vivo infections on vascular function in isolated arteries has not been examined. In pregnancy, systemic and uterine vascular adaptations accommodate increased blood volume through several mechanisms, including decreased sensitivity to vasoconstrictors and increased production of endothelial-dependent vasodilators. We hypothesized that an active in vivo CMV infection would reduce vasodilatation of isolated arteries to the endothelial-dependent vasodilator methacholine and increase vasoconstriction to the α1-adrenergic receptor agonist phenylephrine and that these CMV-induced changes would be accentuated in late pregnancy. A mouse CMV infection model was used to study vascular responses in isolated mesenteric and uterine arteries from nonpregnant and late pregnant mice. In the mouse, CMV is not transmitted to the fetus. Accordingly, there was no evidence of active infection in any fetus examined, even though an active infection was found in salivary glands, uterine and mesenteric arteries, and placentas. Contrary to our hypothesis, increased endothelial-dependent vasodilatation was found in mesenteric arteries from infected compared with uninfected nonpregnant and pregnant mice. These data implicate active CMV infections in hypertensive disorders. Similarly, increased vasodilatation was found in uterine arteries from infected vs. uninfected nonpregnant mice. However, this was completely reversed in infected compared with uninfected late pregnant mice in which vasodilatation in uterine arteries was significantly reduced. Uterine arteries from infected pregnant mice also showed increased vasoconstriction to phenylephrine. Maternal infection led to decreased placental weights but had no effect on fetal weights in late pregnancy. These novel data demonstrate abnormal systemic and uterine vascular responses during an active CMV infection in both nonpregnant and late pregnant mice. Importantly, despite reduced placental weights, fetal weights were maintained, suggesting effective intrauterine compensation in the mouse model.

pregnancy; uterine arteries; mesenteric arteries; vasodilatation; pressure myography

HUMAN CYTOMEegalovirus (HCMV) is a ubiquitous, species-specific DNA virus of the Herpesviridae family. It is endemic, with infection rates in urban populations of 40–80% (14). After the initial primary infection, HCMV enters cycles of dormancy (latency) and reactivation (48). Reactivation occurs in response to stressful (32) or inflammatory (24) situations. HCMV has been implicated in several cardiovascular disorders, including atherosclerosis, coronary heart disease, and cardiac transplant arteriopathy (15, 17, 42). Studies suggest an association of HCMV with cardiovascular disorders through impaired endothelial nitric oxide synthase function (44, 54), leading to endothelial dysfunction (17, 42), measured in vivo as reduced dilation. However, direct links between HCMV, endothelial dysfunction, and vascular diseases remain undefined, and the effect of in vivo infections on vascular function in isolated arteries has not been examined. In this study, a mouse model of acute in vivo mouse cytomegalovirus (mCMV) infection is used to examine the vascular responses of isolated systemic and uterine arteries.

Pregnancy is associated with increased plasma volume, increased cardiac output and decreased systemic vascular resistance. Blood flow to the uterine vascular bed in human pregnancy increases 50- to 70-fold (40, 56). Along with vasculogenesis and angiogenesis, decreased systemic vascular resistance occurs through reduced sensitivity to vasoconstrictors (23, 29, 38) and increased endothelial-dependent vasodilatation by prostacyclin, nitric oxide, and endothelial-derived hyperpolarizing factors (29), which act directly on underlying vascular smooth muscle cells. These vascular adaptations act together to maintain normal blood pressure.

Failure of the systemic and uterine vasculature to adapt during pregnancy leads to several complications, including preeclampsia (30, 43) and intrauterine growth restriction (IUGR) (7, 34). Preeclampsia is diagnosed as the de novo onset of hypertension (blood pressure >140/90 mmHg) with proteinuria after 20-wk gestation; however, other characteristics include an enhanced inflammatory state, platelet activation, increased vasoactivity, generalized vasoconstriction, and reduced organ perfusion (16). IUGR is a significant complication of pregnancy, with both short-term and long-term consequences (39). Reduced uterine blood flow leads to IUGR in animal models (2, 45) and in humans (27). Even modest reductions of uterine blood flow in a sheep model (<25%) lead to IUGR (27). These data underline the importance for normal vascular adaptations in both systemic and uterine arteries during pregnancy. HCMV is associated with pregnancy complications, including preeclampsia (17, 50) and IUGR (4, 50); however, it is difficult to determine if IUGR results from direct fetal infections or indirectly through maternal or placental vascular complications. To circumvent this problem, we used a mouse model of cytomegalovirus (CMV) infection, which reportedly does not cross the placenta (25).

We hypothesized that an active in vivo CMV infection will increase vasoconstriction and decrease vasodilation in systemic and uterine arteries from nonpregnant (NP) female mice. We further hypothesized that these impaired vascular responses will be more evident in pregnancy and will lead to IUGR. We show for the first time that, contrary to our hypothesis, an acute
mCMV infection in both NP and pregnant mice leads to increased vasodilatation in mesenteric arteries. In contrast, we found increased vasoconstriction and decreased vasodilatation in uterine arteries from mCMV-infected late pregnant (LP) mice that leads to decreased placental weights but an increased fetal-to-placental ratio.

**METHODS**

*Animal and tissue preparation.* Virgin C57Bl/6J female mice (2-4 mo of age) were purchased from Jackson Laboratories and housed in Health Sciences Laboratory Animal Services at the University of Alberta. They were infected with mCMV with a lacZ insertion in the expendable immediate early 2 gene (47) (RM427+; gift from E. Mocarski, Stanford University, Stanford, CA). RM427+ was propagated in cultured mouse fibroblasts, and 10⁶ plaque-forming units (PFU) were given to NP mice by intraperitoneal injection, as previously described (47). Infected NP mice were euthanized by cervical dislocation 1-3 wk after infection (NP; n=21) or bred 5-12 days after injection to male C57Bl/6J mice (n=12) to ensure that all infected mice were used within 1 mo of infection. Since infectious virus is still detectible in this time frame (52), it is unlikely that the results of this study were influenced by differences in length of time between viral injection and euthanization. Controls were age-matched uninfected NP (n=33) or pregnant (n=14) mice. Pregnant mice were euthanized on day 17 (D17) or D18; delivery normally occurs on D19 in C57Bl/6J mice. No differences were observed for any vascular response measured between D17 (n=4) and D18 (n=22) pregnant mice, and these data were, therefore, combined. These animal protocols were examined and approved by the University of Alberta Animal Welfare Committee and found to follow the guidelines outlined by the Canada Council of Animal Care.

The salivary glands, uterus, and mesentery were isolated from each mouse and immediately placed in cold HEPES-buffered physiological saline solution (HEPES-PSS; 10 mM HEPES, 1.56 mM CaCl₂, 142 mM NaCl, 4.7 mM KCl, 1.18 mM KH₂PO₄, 1.17 mM MgSO₄, and 5.5 mM glucose at pH 7.5). Maternal weights were recorded daily for each pregnant mouse from D5 to D18 of pregnancy. The fetuses and placentas were isolated from pregnant mice only at D18 and dissected free of fetal membranes and uterine tissue, and the weights were recorded.

*LacZ expression.* Salivary glands, fetuses, and placentas from five infected and uninfected pregnant mice were fixed and stained using the LacZ Detection Kit for Tissues (Invivogen, San Diego, CA), according to the manufacturer’s instructions. The LacZ gene, inserted in the dispensable mCMV immediate early 2 gene, produces β-galactosidase when the virus is actively replicating. β-Galactosidase activity is visualized by the conversion of its substrate, X-gal, to yield a blue product. Photomicrographs were immediately taken.

*Immunofluorescence.* Tissues containing mesenteric and uterine arteries from three infected and uninfected pregnant mice were fixed in 4% formaldehyde overnight, washed with three 5-min washes of phosphate-buffered saline (PBS), and further incubated in 30% sucrose overnight. Tissues were then embedded into OCT embedding medium, snap-frozen in liquid nitrogen, and stored at −80°C. The frozen tissues were sliced on a cryostat in 7- to 10-μm sections, mounted onto glass slides, dried overnight, and stored at −80°C. Before staining, slides were thawed and dried for 1-2 h, and fixed in cold methanol for 10 min at −20°C. Slides were immediately rinsed with three 10-min washes of PBS. Sections were then separated with a PAP pen and blocked with 50 μl of 10% normal goat serum (Cedarlane) in PBS for 3 h. After removing the blocking agent, 50 μl of the primary antibody or blocking agent (antibody diluent; negative control) were added to each section and incubated at 4°C overnight. The primary antibodies used were rabbit anti-Von Willebrand factor (1 μg/ml; Sigma) and chicken anti-β-galactosidase (1 μg/ml; Abbcam).

Following three 5-min washes with PBS, each section was incubated with Alexa Fluor-594 goat anti-rabbit (10 μg/ml) and Alexa Fluor-488 goat anti-chicken (4 μg/ml) secondary antibodies (Molecular Probes, Eugene, OR) for 45 min in the dark. After three 5-min washes with PBS, 4,6-diamidino-2-phenylindole (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. Coverslips were sealed to the slides, which were then stored in the dark at 4°C. Stained sections were viewed with an Olympus IX81 fluorescent microscope (Olympus) using Slidebook 2D, 3D Timelapse Imaging Software (Intelligent Imaging Innovations).

*Myograph studies.* Second-order mesenteric and main uterine arteries were dissected free of adipose and connective tissue. In some cases, both artery types were isolated from the same animal; however, in other cases, separate animals were used for each artery type. In a dual-chamber arteriograph (Living Systems Instrumentation, Burlington, VT), one end of an artery was mounted and tied onto a glass cannula (80- to 100-μm diameter), which was connected to a pressure transducer, to modulate the intraluminal pressure through a servo-controlled peristaltic pump, as previously described (21). Residual blood was flushed out of the 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 and closed off with a valve to hold pressure and prevent flow. The cannulated vessels were held in a 2.5-ml bath containing HEPES-PSS that was replaced every 10 min before and after the dose-response curves. The bath temperature was maintained at 37°C, and the vessels were pressurized to 60 mmHg (mesenteric arteries) or 50 mmHg (uterine arteries) for 30 min. These pressures have been previously reported as optimal for vascular responses in these arteries (18, 37, 49). Arteries unable to maintain pressure were discarded, and new vessels from those same animals were isolated and mounted.

*Experimental design.* Following equilibration, initial lumen diameter was assessed via a charge-coupled device video camera module (Sony) connected to a compound microscope. Lumen diameter was measured digitally using a video dimension analyzer. Vasoconstriction in mesenteric and uterine arteries was assessed using a dose response to the following drugs: 10 nM to 10 μM of phenylephrine (PE; Sigma), an α₁-adrenergic agonist, or 0.01 nM to 1 μM U-46619, a thromboxane mimetic (Calbiochem). Arteries that did not constrict to PE or U-46619 were discarded, and new arteries from that same animal were isolated and mounted. Vasodilation was assessed by preconstricting to ~50% with 40 nM U-46619 (mesentery) or 0.3 μM PE (uterine), followed by a dose response to methacholine (ME; 1 nM to 10 μM; Sigma) to stimulate endogenous production of nitric oxide by endothelium. Following a thorough washout with Ca²⁺÷-free EGTA PSS (10 mM HEPES, 142 mM NaCl, 4.7 mM KCl, 1.18 mM KH₂PO₄, 1.17 mM MgSO₄, 2 mM EGTA) and a 10-min incubation with 100 μM of papaverine (Sigma), passive lumen diameter was recorded. The percent constriction was calculated as 1 - L2/L1 × 100; where L1 is the initial lumen diameter, and L2 is the arterial lumen diameter post-drug addition. The percent dilation was calculated as L2-L1/ L1 × 100 and normalized to the vessel diameter when fully relaxed (passive lumen diameter).

*Statistics.* Data collected were averaged by group (infected and uninfected NP and LP; mesentery and uterine) and treatment. Data that clearly fell at least two standard deviations from the mean were not included in the analysis (<5%, which did not differ between animal groups). Vascular data were compared with the repeated-measures two-way ANOVA followed by Holm-Sidak post hoc analysis to determine significance (P < 0.05). Differences in fetal and placental weights and ratios were determined by taking the mean of the average values from each litter and comparing the two groups with a t-test (P < 0.05).
RESULTS

LacZ expression in tissues from mCMV-infected and uninfected NP and LP mice. An active mCMV infection is confirmed in both NP and LP mice with positive staining for β-galactosidase activity in all salivary glands from infected mice (data not shown). In LP mice, no β-galactosidase activity is detected in any of the fetuses examined (Fig. 1). However, there is greater β-galactosidase activity detected on the maternal (Fig. 1B) compared with fetal (inset) side of the placenta from infected LP mice. The endothelial and vascular smooth muscle cells of uterine arteries from mCMV-infected mice are strongly positive for the β-galactosidase enzyme compared with uninfected cells for both LP (Fig. 2) and NP (data not shown) mice. Similar results were found in mesenteric arteries from both LP and NP mice (data not shown).

Passive lumen diameters of mCMV-infected and uninfected mesenteric and uterine arteries. Passive lumen diameters in mesenteric arteries from uninfected mice increase in LP compared with NP (LP = 223 ± 9.7 μm, NP = 189 ± 5.1 μm; P < 0.01). This adaptive response to pregnancy in mesenteric arteries is absent in mCMV-infected mice (LP = 217 ± 4.7 μm, NP = 209 ± 8.2 μm; nonsignificant). This is likely due to the significantly increased passive lumen diameter in infected vs. uninfected NP mice (P < 0.05) with no corresponding increase in infected compared with uninfected LP mice. In uterine arteries, passive lumen diameters in LP vs. NP mice are significantly increased in mCMV-infected uterine arteries (LP = 287 ± 18.3 μm, NP = 187 ± 11.8 μm; P < 0.01) similarly to uninfected uterine arteries (LP = 283 ± 13.0 μm, NP = 173 ± 5.9 μm; P < 0.01). There is no significant difference in NP or LP uterine arteries when infected are compared with uninfected in each group.

PE-induced vasoconstriction in mesenteric and uterine arteries from mCMV-infected and uninfected NP and LP mice. The vasoconstriction response to increasing concentrations of the α1-adrenergic receptor agonist PE is not significantly different in mesenteric arteries isolated from infected compared with uninfected NP mice (Fig. 3A). However, surprisingly, there is a significant decrease in PE-induced vasoconstriction in mesenteric arteries from infected compared with uninfected LP mice (P < 0.05; Fig. 3B).

In uterine arteries from NP mice, there is no difference in PE-induced vasoconstriction between infected and uninfected mice (Fig. 4A). However, in contrast to the mesenteric arteries, vasoconstriction to PE in uterine arteries from LP infected mice is significantly greater (P < 0.05) than the response in uterine arteries from LP uninfected mice (Fig. 4B). In this animal model, the PE response is not significantly reduced in uterine arteries from LP compared with NP mice, regardless of mCMV infection status (compare Fig. 4, A and B).

ME-induced vasodilation in mesenteric and uterine arteries from mCMV-infected and uninfected NP and LP mice. Although a dose response to PE could be evaluated in mesenteric arteries, the vasoconstriction was not stable for longer than 25 min and so could not be used for preconstriction in vasodilation studies. In addition, there was a significant difference in the dose required for 50% vasodilation in LP-infected mice (Fig. 3B). A thromboxane mimetic, U-46619, was, therefore, used as a preconstrictor for mesenteric arteries. To confirm that mCMV does not affect responses to this vasoconstrictor, a dose-response curve for U-46619 was done on the mesenteric arteries. In both NP and LP mice, there are no significant differences in U-46619-induced vasoconstriction between infected and uninfected mice (Fig. 3, C and D).

The vasodilation response to increasing concentrations of the endothelial-dependent vasodilator, ME, was measured in mesenteric and uterine arteries isolated from infected and uninfected NP and LP mice. Interestingly, in mesenteric arteries, there is a significant increase (P < 0.05) in ME-induced vasodilation in infected NP and LP compared with uninfected NP and LP mice, respectively (Fig. 5A). Mesenteric arteries from mCMV-infected NP mice showed the greatest ME-induced vasodilation (P < 0.05). The ME-induced vasodilation is lowest and not significantly different in mesenteric arteries from uninfected LP vs. NP mice (Fig. 5A).

Similarly, ME-induced vasodilation in uterine arteries from infected vs. uninfected NP mice is significantly increased (P < 0.05; Fig. 5B). In late pregnancy, this ME-induced vasodilation is significantly decreased in uterine arteries from infected compared with uninfected mice (P < 0.05). There is no significant difference in the normal ME response between uninfected LP and NP mice (Fig. 5B); however, there is a dramatic reduction in ME-induced vasodilation in infected LP mice compared with infected NP mice (Fig. 5B).

Fig. 1. Active mouse cytomegalovirus (mCMV) infection in placentas from late pregnant (LP) mCMV-infected mice. LacZ expression detected through β-galactosidase activity (blue) was used to confirm an active mCMV infection. Staining was performed on the fetal (inset) and maternal side of the placenta uninfected LP mouse (A) and the fetal (inset) and maternal side of the placenta from mCMV-infected LP mouse (B).
Fetal and placental weights from mCMV-infected and uninfected D18 pregnant mice. Litter size and fetal and placental weights were compared between infected and uninfected D18 pregnant mice. There is no significant difference in fetal weights (Fig. 6A); however, placental weights are reduced ($P < 0.05$) and the fetal-to-placental ratio is increased ($P < 0.05$) in mCMV-infected LP mice (Fig. 6, B and C). There is no significant difference in litter size or maternal weights throughout pregnancy between mCMV-infected and uninfected LP mice (data not shown).

DISCUSSION

This is the first study to show that an active in vivo CMV infection results in vascular dysfunction in both NP and LP mice. Mouse CMV did not cross the placenta to infect the fetus; however, an active infection was demonstrated in uterine and mesenteric arteries and was more evident on the maternal compared with fetal side of the placenta. Contrary to our hypothesis, mesenteric arteries, which are representative of systemic resistance arteries, showed significantly increased ME-induced vasodilation when isolated from infected compared with uninfected mice, and this occurred in both NP and LP mice. Similar results were found in uterine arteries from NP mice. However, in keeping with our hypothesis, uterine arteries from LP mice showed decreased vasodilation to ME and increased vasoconstriction to PE. Although placental weights were reduced, there was no effect on fetal weights. Thus these data demonstrate that an active maternal CMV infection im-

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**Fig. 2.** Active mCMV infection in uterine arteries from LP mCMV-infected mice. To confirm an active mCMV infection in the uterine arteries and surrounding tissue, sections of uterine tissue isolated from LP mCMV-infected (A–C) and uninfected (D–F) mice were stained for β-galactosidase (green; A and D), Von Willebrand factor to detect endothelium (red; B and E), or both (C and F). All nuclei were stained with 4,6-diamidino-2-phenylindole (blue).
pairs maternal vascular function and adversely affects placental
development in pregnancy.

Much research has surrounded congenital CMV infections
(4); however, less is known about CMV-induced pregnancy
complications independently of a congenital infection. An
important aspect of this study in the mouse model is that it
shows the effect of an active maternal infection without the
influence of a congenital infection (25). The lack of mCMV
transmission to the fetus was confirmed by the absence of
β-galactosidase activity in the fetus.

Contrary to our hypothesis, mesenteric arteries from mCMV-
infected NP and LP mice responded with greater vasodilation to
ME than uninfected mice. It is interesting to contemplate a role
for mCMV in hypotension, since hypotension occurs in several
diseases, including diabetes (31), autonomic neuropathy (3),
septic shock (36), and dementia in aging (33) in which CMV
infections could play a part (1, 28, 51). In pregnancy, the
consequences of chronic hypotension are reduced birth weight
and preterm delivery, likely as a result of reduced uteroplacen-
tal blood flow from reduced perfusion pressure (19, 34). In
support of our hypothesis, uterine arteries from mCMV-in-
fected pregnant mice showed decreased ME-induced vasodilata-
tion and increased PE-induced vasoconstriction. This, in con-
junction with the increased ME-induced vasodilation in mes-
enteric arteries and potential reduction in perfusion
pressure, could lead to decreased blood flow to the placenta
and fetus and IUGR. However, although placental weights
were significantly reduced as might be expected, there was
no change in fetal weights, leading to an increased fetal-to-
placental ratio. This has been previously found in gesta-
tional hypertension, where IUGR is not always seen with
reduced placental perfusion (55). This suggests that there is
increased placental efficiency in nutrient and oxygen trans-
port to compensate for the impaired vascular responses and
reduced placental size in C57Bl/6J mice, which are rela-
tively resistant to mCMV (20); however, the effects of
mCMV infection on length of gestation, newborn weights,
or pregnancy outcomes in more susceptible mouse strains

Fig. 3. Vasoconstriction to phenylephrine (PE) and U-46619 in mesenteric arteries from nonpregnant (NP) and LP mCMV-infected and uninfected mice.
Changes in lumen diameter were measured in response to increasing concentrations of PE (A and B) or U-46619 (C and D) in mesenteric arteries from
mCMV-infected and uninfected NP mice (A and C) and mCMV-infected and uninfected LP mice (B and D). Data were expressed as the mean ± SE percent
decrease in arterial lumen diameter at each dose of PE or U-46619 compared with the lumen diameter before drug addition. *Significant difference between points
on the curves was determined by repeated-measures two-way ANOVA with Holm Sidak post hoc (P < 0.05).
such as Balb/c are not yet known. As well, given the evidence of increased infection on the maternal compared with fetal side of the placenta, it is possible that mCMV-induced changes in placental morphology may contribute to reduced placental weights in addition to the observed impaired vascular responses.

The impaired vascular responses observed in this study may be a direct effect of CMV infection in the vascular wall (5), or an indirect effect via a viral-induced inflammatory response (57). A fully productive infection or simply viral attachment alone (without internalization or viral replication) stimulates the cell to produce prostaglandins, reactive oxygen species, and activation of nuclear factor-kB, leading to production of inflammatory cytokines, all of which can increase vasoconstriction (9, 46, 58). We show that both endothelial and vascular smooth muscle cells in mesenteric and uterine arteries are directly infected with mCMV. It is, therefore, likely that the impaired vascular responses in mCMV-infected mice involve viral-induced changes in endothelial function. A seropositive status for HCMV is associated with endothelial dysfunction and an increased risk of adverse cardiovascular events (15, 17, 42). As well, CMV decreases the activation of endothelial nitric oxide synthase (44, 54) and decreases release of the potent vasodilator nitric oxide. CMV also increases prostaglandin H synthase-2 activity, which is important for viral replication (59); this can lead to production of vasodilators, such as prostacyclin. It is important to note that the balance of factors contributing to endothelial-dependent vasodilation vary considerably among vascular beds and sometimes even within a vascular bed, depending on the location (6, 21). Thus it is

Fig. 4. Vasoconstriction to PE in uterine arteries from NP and LP mCMV-infected and uninfected mice. Changes in lumen diameter were measured in response to increasing concentrations of PE in uterine arteries from mCMV-infected and uninfected NP mice (A) and mCMV-infected and uninfected LP mice (B). Data were summarized and expressed as the mean ± SE percent decrease in arterial lumen diameter at each dose of PE compared with the lumen diameter before drug addition. *Significant difference between the curves was determined by repeated-measures two-way ANOVA with Holm Sidak post hoc (P < 0.05).

Fig. 5. Vasodilation to methacholine (ME) in mesenteric and uterine arteries from NP and LP mCMV-infected and uninfected mice. A: mesenteric arteries were preconstricted with 40 nM U-46619. B: uterine arteries were preconstricted with 0.3 μM of PE. Changes in lumen diameter were measured in response to increasing concentrations of ME in arteries from NP and LP mCMV-infected and uninfected mice. Data were summarized and expressed as the mean ± SE percent increase in arterial lumen diameter at each dose of ME compared with the lumen diameter before drug addition. *Significant difference between the curves was determined by repeated-measures two-way ANOVA with Holm Sidak post hoc (P < 0.05).
essential to further investigate the relative roles of nitric oxide, prostaglandins, and endothelial-dependent hyperpolarizing factors using inhibitors for these pathways to determine the mechanism by which mCMV is inducing differential vascular dysfunction in mesenteric compared with uterine arteries in this in vivo model.

Vascular adaptations to pregnancy include decreased sensitivity to vasoconstrictors (23, 29, 38) and increased vasodilation to balance increased blood volume and to maintain peripheral blood pressure (29). In the mouse, Cooke and Davidge showed increased endothelial-dependent vasodilation in uterine and mesenteric arteries in pregnancy (11). In addition, several studies in rats and guinea pigs have also found a decreased sensitivity to PE and an increased sensitivity to ME in uterine and mesenteric arteries (13, 22, 26, 35, 41, 53), although one study showed an increased sensitivity to PE in uterine arteries (8). In our studies in the uninfected mouse, however, no significant differences in PE or ME responses were found when LP were compared with NP mice in either mesentery or uterine arteries. This is in agreement with a study showing no difference in overall endothelial-dependent vasodilation in mesenteric arteries in pregnancy (10). One explanation for these opposing results is the differing methodology to evaluate vascular function. Most studies used the wire myograph system (13, 26, 35, 41, 53), which allows the drug to access both the endothelium and the vascular smooth muscle cells directly and simultaneously. In our study, the pressure myograph system was used in which the lumen of the artery, and thus the endothelium, is closed off to drugs delivered to the bath; drugs must first pass through the vascular smooth muscle cells to interact with the endothelium. It is possible that this process is reduced in arteries from pregnant mice. A second explanation is differences in artery location, i.e., main compared with radial uterine arteries. In the pregnant rat, D’Angelo and Osol found increased sensitivity to PE in the main uterine artery (12); whereas Hermsteiner et al. found decreased sensitivity to PE in the radial uterine artery (22). Although both studies used the pressure myograph system, different results were found.

Based on our findings that an active CMV infection negatively impacts pregnancy-induced vascular adaptations differently in systemic and uterine arteries and also impairs normal vascular function in the absence of pregnancy, further studies are essential to determine the mechanisms by which this happens. Determining which pathways CMV works through will facilitate the development of valuable pharmaceutical and/or immunological agents for the effective treatment of viral-associated vascular dysfunction. Demonstration of a direct link between an active CMV infection and impaired vascular responses suggests a new paradigm for understanding cardiovascular diseases stemming from vascular dysfunction. These data support the importance of prevention of CMV infections through use of vaccines, particularly in young seronegative women of childbearing age and also in controlling an active CMV infection in pregnancy.

ACKNOWLEDGMENTS

We thank Dr. E. Mocarski (Stanford University) for the gift of RM427+, the mCMV virus used in our studies. We also thank Rana Haidar and Maggie Wang for technical assistance.

GRANTS

Funding support is acknowledged from Natural Sciences and Engineering Research Council of Canada and the Canadian Institutes of Health Research-funded Maternal-Fetal-Newborn Health Training Grant.
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