Spiral Arterial Remodeling Is Not Essential for Normal Blood Pressure Regulation in Pregnant Mice

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Abstract—Maternal cardiovascular adaptations occur in normal pregnancy, systemically, and within the uterus. In humans, gestational control of blood pressure is clinically important. Transient structural remodeling of endometrial spiral arteries normally occurs in human and mouse pregnancies. In mice, this depends on uterine natural killer cell function. Using normal and immune-deficient mice, we asked whether spiral artery remodeling critically regulates gestational mean arterial pressure and/or placental growth. Radiotelemetric transmitters were implanted in females and hemodynamic profiles to a dietary salt challenge and to pregnancy were assessed. Implantation sites from noninstrumented females were used for histological morphometry. Both normal and immune-deficient mice had normal sensitivity to salt and showed similar 5-phase gestational patterns of mean arterial pressure correlating with stages of placental development, regardless of spiral artery modification. After implantation, mean arterial pressure declined during the preplacental phase to reach a midgestation nadir. With gestation day 9 opening of placental circulation, pressure rose, reaching baseline before parturition, whereas heart rate dropped. Heart rate stabilized before parturition. Placental sizes deviated during late gestation when growth stopped in normal mice but continued in immune-deficient mice. As an indication of the potential for abnormal hemodynamics, 2 pregnant females delivering dead offspring developed late gestational hypertension. This study characterizes a dynamic pattern of blood pressure over mouse pregnancy that parallels human gestation. Unexpectedly, these data reveal that spiral artery remodeling is not required for normal gestational control of blood pressure or for normal placental growth. (Hypertension. 2010;55:729-737.)

Key Words: blood pressure monitoring  ■  hemodynamics  ■  lymphocytes  ■  mice  ■  pregnancy  ■  remodeling

Well-defined, systemic cardiovascular adaptations occur during normal pregnancy. These include significant increases in heart rate, cardiac output, and blood volume. These changes often accompany physiological cardiac hypertrophy, which, in nonpregnant individuals, would lead to hypertension. In pregnancy, however, despite blood volume gain, mean arterial pressure (MAP) is maintained or declines. This gestational antihypertensive mechanism is postulated to involve systemic vasodilation, as well as transient insensitivity of the vasculature to vasoactive substances (eg, products of the renin-angiotensin system). Hemodynamic alterations occur during the first trimester of human pregnancy, before completion of placental development, and are thought to be critical for the support of the high metabolic demands of a growing fetus.1

Regional and local structural changes that ensure adequate perfusion of the early maternal-fetal interface are also required for pregnancy success. These are less well defined. In humans, rats, and mice, uterine arteries and veins undergo substantial outward circumferential remodeling. Coincidentally, the endometrial vascular bed enlarges via vasculogenesis and angiogenesis, supporting endometrial decidualization and uterine enlargement. Spiral arteries (SAs), the major vessels participating in endometrial remodeling at implantation sites, arise from the uterine arteries. SAs begin as high-resistance, low-capacity arteries and remodel to low-resistance, high-capacity venous-like structures.2,3 In women, SA remodeling is initiated by invasive trophoblast and uterine natural killer (NK; uNK) cells and is completed by midgestation.3,4 In mice, uNK cells are solely responsible for SA modification.5 uNK cells are a specialized, terminally differentiated NK cell subset that is poorly cytotoxic but secretes large amounts of angiogenic, chemotactic, and inflammatory cytokines.6

Important human pregnancy complications are linked with inadequate local vascular adaptations, including fetal loss,7 intrauterine growth restriction, gestational hypertension, and preeclampsia (PE).8,9 We sought to determine the relative importance of physiological remodeling of SAs on systemic hemodynamics of pregnant mice using continuous assess-
ment by radiotelemetry and on growth of the placenta subsequent to SA remodeling. We compared hemodynamic and placental morphometric data from normal mice with NK-cell, T-cell, B-cell deficient (Rag2\(^{-/-}\) γc\(^{-/-}\); immune deficient) mice that lack SA remodeling. Neither gestational hypertension nor deficient placental growth was an outcome of impaired SA remodeling.

**Methods**

**Animals**

C57BL/6J mice (B6) were purchased from the Jackson Laboratory (Bar Harbor, ME) and conventionally housed. BALB/cAnNCrl mice (BALB/c) were purchased from Charles River Canada and housed under barrier husbandry. BALB/c-Rag2\(^{-/-}\) γc\(^{-/-}\) mice were bred under barrier husbandry at Queen’s University from pairs generously donated by Dr Mamuro Ito (Central Institute for Experimental Animals, Kawasaki, Japan). All of the mice had free access to food and water and entered studies at 10 weeks of age. Animal usage complied with protocols approved by the Queen’s University Animal Care Committee.

**Telemetry Implant Surgery and Data Acquisition**

TA11PA-C10 radiotransmitters (Data Sciences International) were implanted via the common carotid, as reported by others.\(^{10}\) Briefly, weighed virgin females were anesthetized with isoflurane. The cervical ventral midline was incised (2 cm), and the submandibular glands were separated with sterile cotton swabs. The left common carotid artery was visualized, retracted, and temporarily occluded with a microvessel clamp. The artery was punctured with a 26-gauge needle, the catheter tip of the transmitter was advanced to the aortic arch using cannulation forceps, and the catheter was sutured in place. For the salt challenges, immune-competent controls (allogenic B6, C57BL/6J mice (B6) were purchased from the Jackson Laboratory (Bar Harbor, ME) and conventionally housed. BALB/cAnNCrl mice (BALB/c) were purchased from Charles River Canada and housed under barrier husbandry. BALB/c-Rag2\(^{-/-}\) γc\(^{-/-}\) mice were bred under barrier husbandry at Queen’s University from pairs generously donated by Dr Mamuro Ito (Central Institute for Experimental Animals, Kawasaki, Japan). All of the mice had free access to food and water and entered studies at 10 weeks of age. Animal usage complied with protocols approved by the Queen’s University Animal Care Committee.

**Study Protocol**

Hemodynamic data were collected for 30 seconds every 4 minutes. For the salt challenges, immune-competent controls (allogenic B6, n = 5; congenic BALB/c, n = 4) and Rag2\(^{-/-}\) γc\(^{-/-}\) (n = 4) were fed 4 days each with diets containing normal salt (0.67%), low salt (0.43%), high salt (8.50%), and then normal salt (Purina). Food consumption, water intake, and body weight were measured daily. Then, females were selected for estrus and paired with genetically matched males for timed matings. No data were collected during lactation. C57BL/6J mice (B6) were purchased from the Jackson Laboratory (Bar Harbor, ME) and conventionally housed. BALB/cAnNCrl mice (BALB/c) were purchased from Charles River Canada and housed under barrier husbandry. BALB/c-Rag2\(^{-/-}\) γc\(^{-/-}\) mice were bred under barrier husbandry at Queen’s University from pairs generously donated by Dr Mamuro Ito (Central Institute for Experimental Animals, Kawasaki, Japan). All of the mice had free access to food and water and entered studies at 10 weeks of age. Animal usage complied with protocols approved by the Queen’s University Animal Care Committee.

**Histology**

For placental morphometry, nontransmitter-implanted Rag2\(^{-/-}\) γc\(^{-/-}\) (n = 3 per time point) and immune-competent BALB/c (n = 3 per time point) females were deeply anesthetized at gd10, gd12, gd14, gd16, and gd18 using tribromoethanol (250 mg/kg) before euthanasia (cervical dislocation) and dissection. For SA morphometry, other gd12 females were perfused with 30 mL of 4% neutral buffered paraformaldehyde (Sigma-Aldrich) with 0.1 mol/L of sucrose (Sigma; pH 7.4). Uteri were removed after 30 mL of paraformaldehyde. Tissues were fixed (nonperfused samples) or postfixed (perfused samples) for 6 hours in PFA, rinsed in 70% ethanol, processed automatically into paraffin, and embedded using routine methods.\(^{11}\) Implantation sites were serial sectioned at 7 μm and stained with hematoxylin/eosin. To estimate placental size, 3 placentas per dam were scored, 15 sections from each placenta, selecting the center (largest) section and moving outward. SAs were scored as described previously.\(^{12}\) Lumen diameters (LDs) were derived from circumferential (C) determinations for each vessel cross-section, as follows: LD = C/π. Wall thicknesses were calculated as follows: (wall diameter – LD)/2. Calculations were made using Zeiss AxioVision Software (version 4.6).

**Statistics**

Data were analyzed using Prism 4.03 Statistical Software (GraphPad) and are presented as mean±SEM. All of the hemodynamic data were analyzed using 24-hour means. Salt-challenge data were analyzed using the lowest-day MAP for each animal on low salt and the highest-day MAP on high salt. Because MAP from gd10 to gd3 was stable in preliminary studies, this MAP was averaged to define baseline, and each subsequent gd was compared to obtain the Δvalue. Data were analyzed by paired 1-way repeated-measures ANOVA with a Dunnett post hoc test within strain and 1-way ANOVA with Bonferroni post hoc test between strains. When sample sizes were unequal, Bartlett correction was used. Morphometric measurements were compared between groups using 2-tailed t tests. P<0.05 was considered significant.

**Results**

**Dietary Salt Challenge**

Baseline MAP was not different among B6 (109.1±1.26 mm Hg), BALB/c (113.0±1.41 mm Hg), or Rag2\(^{-/-}\) γc\(^{-/-}\) mice (116.4±1.28 mm Hg). Dietary salt challenge indicated that arterial pressures of unmated females of each genotype were similarly sensitive to changes in dietary salt. All of the mice showed a decrease in MAP with low salt that did not differ between genotypes: B6 −4.8±0.88 mm Hg; BALB/c −4.8±1.31 mm Hg; and Rag2\(^{-/-}\) γc\(^{-/-}\) −5.7±1.74 mm Hg. Similarly, the 3 strains exhibited increases in MAP with salt loading: B6 8.3±1.30 mm Hg; BALB/c 7.1±1.95 mm Hg; and Rag2\(^{-/-}\) γc\(^{-/-}\) 4.5±0.75 mm Hg (P>0.05 between strains). The amplitude of change in MAP between low- and high-salt diets was not different between B6 and BALB/c or between the genotypes of BALB/c, indicating similar degrees of salt sensitivity: B6 10.2±1.58 mm Hg; BALB/c 13.3±1.70 mm Hg; and Rag2\(^{-/-}\) γc\(^{-/-}\) 11.5±2.18 mm Hg. In addition, no hemodynamic differences were observed between virgin females of these genotypes in any other parameters (SAP, DAP, HR, PP, or activity; not shown).

**Gestational Studies: Spiral Arterial and Placental Morphometry**

We reported previously that pregnant B6 and BALB/c mice experience midgestational SA remodeling, whereas B6-Rag2\(^{-/-}\) γc\(^{-/-}\) mice do not.\(^{13}\) To confirm that pregnant BALB/c-Rag2\(^{-/-}\) γc\(^{-/-}\) mice lack remodeled SAs, gd12 histological studies were undertaken. For remodeled SAs in BALB/c females, the wall:lumen ratio was nearly half of that observed in Rag2\(^{-/-}\) γc\(^{-/-}\) mice (0.21±0.009 versus 0.39±0.020; P<0.0001; Figure 1A). In BALB/c mice, SA lumens were dilated to 90.3±3.57 μm (Figure 1B) compared with 79.1±2.72 μm (P<0.05) in Rag2\(^{-/-}\) γc\(^{-/-}\) mice (12.4%
narrower). Vessel walls in BALB/c mice were thin with minimal smooth muscle (wall thickness: 10.1 ± 0.40 µm; Figure 1C) compared with Rag2/−/−/− mice with thicker muscle walls (16.2 ± 0.55 µm; P < 0.0001) that contained extracellular matrix deposits. On the basis of the Poiseuille law (R = 1/r⁴), the impact of the lack of SA remodeling on vascular resistance (R) was calculated to be 1.7-fold greater in Rag2/−/−/− SAs than in gd-matched immune-competent BALB/c mice.

To determine whether differences in SA modification might alter placental growth and, thus, confound our planned hemodynamic study, a time course study of placental cross-sectional areas was conducted comparing Rag2/−/−/− and BALB/c mice. At gd9.0 to gd9.5, placental development is completed by fusion of the allantois to the chorion (trophoblast component), establishing fetal circulation within the placenta. Thus, midsagittal placental surface areas were measured from gd10 through gd18 (Figure 1D). In both strains, placental growth was equivalent and linear from gd10 to gd14. At gd16, placental surface area was greater in BALB/c than in Rag2/−/−/− mice (6.16 ± 0.17 mm² versus 5.40 ± 0.14 mm²; P < 0.001). Between gd16 and gd18, BALB/c placentas showed no growth, whereas Rag2/−/−/− placertas exhibited further growth. At gd18, BALB/c placentas were smaller than those of Rag2/−/−/− mice (5.99 ± 0.29 mm² versus 7.02 ± 0.26 mm²; P < 0.01).

**MAP Profile of Pregnant Immune-Competent Mice With Gestational SA Modification**

We used 2 strains of immune-competent mice: B6 to enable comparisons with data published by others and BALB/c mice congenic to the experimental immune-deficient mice. Continuous data collection enabled detection of subtle hemodynamic changes. After mating, no difference was detected between prepregnancy baseline MAP and that of gd0 to gd3 (preimplantation) for B6 or BALB/c mice. Thus, a gd0 to gd3 MAP was calculated for each group and used as its baseline value for later gestational comparisons. For both strains, MAP remained constant until gd5 and then declined, reaching a nadir at gd9 (Figure 2A and 2B). MAP returned to baseline by gd12 and remained stable until peripartum (gd18). Continuous data are only presented to gd18 because of variation in the timing of parturition between females. The final day of study that included parturition and maternal care until 7:00 AM are presented as “P” in the figure. Statistical comparisons are not reported for the 24 hour including parturition. MAP values were not statistically different between B6 and BALB/c mice at any gd. In addition, there were
MAP Profile in Pregnant Immune Deficient Mice Lacking SA Modification

Pregnant Rag2\(^{-/-}\)y\(^{c-/-}\) mice exhibited a nearly identical MAP profile to that of immune-competent mice (Figure 2C). Specifically, in pregnant Rag2\(^{-/-}\)y\(^{c-/-}\) mice, MAP was stable until gd5, after which a small but statistically significant decline in MAP occurred between gd6 and gd9 (gd6: \(-2.04 \pm 0.72; \) gd9: \(-5.57 \pm 0.57; \) \(P<0.01\)). MAP then increased back toward baseline by gd12 and was relatively stable until just before parturition. No statistically significant differences were found in MAP profiles between Rag2\(^{-/-}\)y\(^{c-/-}\) and immune-competent mice.

HR During Mouse Pregnancy

Figure 2D presents gestational HR profiles for immune-competent strains and Rag2\(^{-/-}\)y\(^{c-/-}\) mice. The HR pattern of pregnant immune-competent mice was relatively constant to gd4, rose (+25 to 30 bpm) to peak values between gd7 and gd9, and then declined until parturition. HR was slightly lower in Rag2\(^{-/-}\)y\(^{c-/-}\) mice than in immune-competent mice (significantly different only at gd7; \(P<0.05\)). Despite this, HR declines similarly in both groups beginning at midgestation. This decrease in HR from is \(-25\) bpm in both groups during late gestation. The decline in Rag2\(^{-/-}\)y\(^{c-/-}\) HR was significant compared with baseline at gd13, gd16, and gd17 (\(P<0.05\)).

SAP, DAP, and PPs During Mouse Pregnancy

The general trends of gestational profiles of SAP and DAP in immune-competent and Rag2\(^{-/-}\)y\(^{c-/-}\) mice were similar to MAP (Figure 3A and 3B). For immune-competent mice, SAP declined significantly at gd8 to gd9 (\(-6\) to \(7\) mm Hg; \(P<0.01\)) but returned to baseline by gd12. Changes in DAP were of less magnitude at these times. For Rag2\(^{-/-}\)y\(^{c-/-}\) mice, SAP declined significantly. At gd9, SAP had dropped 6 to \(7\) mm Hg (\(P<0.001\)), whereas DAP had fallen 4.50 \(\pm\) 0.51 mm Hg (\(P<0.001\)). As in immune-competent mice, both values returned to baseline at gd12. There were no differences in SAP or DAP between immune-competent and Rag2\(^{-/-}\)y\(^{c-/-}\) mice at any time point.

PP, a more recent predictor of cardiovascular risk, had a similar pattern in immune-competent and Rag2\(^{-/-}\)y\(^{c-/-}\) mice (Figure 3C). In general, early gestational PP was stable; there was a small, significant decline (2 to 3 mm Hg) between gd8 and gd9 and a return to baseline by gd12. No differences between groups were detected at any time point.

Activity During Mouse Gestation

Others have demonstrated that mutations in the mouse Rag2 gene result in fatigue and lower activity than in control animals.\(^{14}\) In our immune-competent mice, activity was relatively constant during the gd0 to gd3 baseline and then rose to a peak between gd5 and gd7 (Figure 4). After this, activity declined slowly, passing baseline at gd12, and reaching a statistically significant lower level at gd16 to gd17 (\(-30\%\); \(P<0.05\)). This pattern is identical to that of HR in immune-competent mice, coincident with the timing of increased HR and activity. Rag2\(^{-/-}\)y\(^{c-/-}\) mice showed less activity early in pregnancy and then declined at a similar rate from gd8 as in immune-competent mice to reach \(-40\%\) at gd16 to gd17 (\(P<0.001\)).
Reproductive Outcomes and Complications

Birth data for the animals in this study, including pups found dead, were 4.9±0.7 Rag2\(^{-/-}\)γc\(^{-/-}\) pups (15 litters) and 4.6±0.8 BALB/c pups (5 litters; \(P>0.05\)). Rag2\(^{-/-}\)γc\(^{-/-}\) pup weights averaged 1.48 g, which is within the normal range for congenic pups (currently, no comparative syngeneic data exist). Previous studies report BALB/c pup weights as 1.39 g for 9 litters\(^{15}\) and 1.53 g for 11 litters\(^{16}\).

Figure 4. Percentage change in activity of immune-competent \(n=9; \bigcirc\) and Rag2\(^{-/-}\)γc\(^{-/-}\) mice \(n=9; \bullet\) across gestation. No gd0 through gd3 baseline activity differences were observed between immune-competent (9.50±1.38) and Rag2\(^{-/-}\)γc\(^{-/-}\) (11.30±1.22) mice. Values are represented as mean±SEM of 24-hour continuous recordings. \(\phi P<0.05\) vs baseline within immune-competent group; \(\phi P<0.05\) vs baseline within Rag2\(^{-/-}\)γc\(^{-/-}\) group.

In addition to surgical and equipment failures, reproductive complications occurred in radiotransmitter-implanted females. As in other mouse populations, some females failed to copulate, whereas others failed to conceive after mating. The latter are endocrinologically pseudopregnant until day 7 after mating, when estrus returns. The pseudopregnancy MAP profile was compared with pregnant Rag2\(^{-/-}\)γc\(^{-/-}\) mice to gd11, when pseudopregnancy was confirmed by the absence of weight gain or palpable fetuses (Figure 5A). In pseudo-pregnancy, MAP was stable, suggesting that postimplantation conceptuses rather than hormonal changes (ie, ovarian effects without a conceptus) account for the early gestational drop in the MAP of pregnant mice \((P<0.05)\).

Dystocia, the inability to vaginally deliver newborns at term, was seen in 2 radiotransmitter-implanted Rag2\(^{-/-}\)γc\(^{-/-}\) females. Loss of condition and mobility onset acutely at gd16 and showed hypertension in later gestation (Figure 5B). These distressed females were euthanized and examined. The litters were 5 and 10, and all of the fetuses, including the ones entered into the pelvic inlet, were of normal size and viable. These females had identical hemodynamic profiles to Rag2\(^{-/-}\)γc\(^{-/-}\) mice with normal pregnancy (Figure 5B), suggesting that there is no prodromal hemodynamic event to dystocia.

Two additional Rag2\(^{-/-}\)γc\(^{-/-}\) mice gave birth to small litters of dead pups. One female gave birth to a single stillborn, whereas the other gave birth to 1 viable and 2 dead pups. MAP profiles for these animals were distinctly different and showed hypertension in later gestation (Figure 5B). No etiology for these late-gestation deaths was found by histological or bacterial analyses (Laboratory Animal Pathology, University of Guelph).

Discussion

This study identified a distinct, 5-phase pattern of arterial pressure in successful pregnancies of 2 distinct strains of normal, immune-competent mice that have SA remodeling (Figure 6). Phase 1 of the pattern had stable arterial pressures...
with prepregnancy baseline hemodynamic values. This gd0 to gd5 interval is the stage of preimplantation development that ends with gd3.75 blastocyst hatching and implantation at \( \sim \)gd4.00. During phase 2 (gd5 through gd9), maternal MAP declined, corresponding with the interval of preplacental development that ends with gd3.75 blastocyst hatching and implantation at \( \sim \)gd4.00. During phase 2 (gd5 through gd9), maternal MAP declined, corresponding with the interval of preplacental development. By this stage, the blastocyst tropho
toderm has differentiated into the placental primordium called the “ectoplacental cone,” and endometrial decidualiza
tion has occurred on the antimesometrial side of the uterus. Decidualization then spreads to the mesometrial side where, at gd6, uNK cells suddenly appear in abundance as rapidly dividing cells.\(^{17}\) A decline in MAP during this early postimplantation/preplacental stage has not been delineated previously in rodents but is known in women.\(^{1}\) Phase 3 onset at gd9 extended to gd14 and coincided with histologically recognized remodeling of SA in immune-competent mice. This is also an interval of placental growth.\(^{18–20}\) During phase 3, MAP rose gradually back toward the prepregnancy baseline, whereas HR declined. In Phase 4 (gd14 through gd18), a time of rapid fetal growth after placental maturation, these patterns of change continued at a more modest rate.\(^{18–20}\) Phase 5 covered the peripartum interval, which was not remarkably distinct.\(^{21}\) Similar profiles were found during radiotelemetry-monitored second pregnancies in study animals (data not shown).

Unexpectedly, the pattern of gestational MAP in \( \text{Rag}^2{-}^{-}\gamma_c{-}^{-}\) mice, which do not undergo SA remodeling, was indistinguishable from normal mice. Although we estimated that the lack of remodeling of individual SAs increased vascular resistance in \( \text{Rag}^2{-}^{-}\gamma_c{-}^{-}\) implantation sites by 60% relative to those in normal females, pregnant \( \text{Rag}^2{-}^{-}\gamma_c{-}^{-}\) females had no predisposition toward hypertension, placenta
growth restriction, or fetal loss. This suggests that, under normal conditions, the upstream maternal vasculature can compensate for inadequate SA remodeling without causing placental insufficiency. This outcome likely results from the absence of lymphocyte responses, which, in human placental insufficiency, are thought to promote strong type 1 inflammatory responses and cell killing. Although an inadequately remodeled SA may be a predisposing factor to placental insufficiency, it does not itself trigger hypertension. Our data support a novel distinction between SA remodeling versus placental signals as the initiating factor for gestational hyper
tension. Placental insufficiency and/or conceptus stress sig
nals initiate downstream effectors. Placental tissues release microparticles, angiogenic and vasoactive compounds, che
mokines, and cytokines (vascular endothelial growth factor, placental growth factor, and soluble fms-like tyrosine kinase 1), which all act on the maternal vasculature to restrict the volume of SA flow and to further exacerbate placental hypoxic and/or reperfusion injury. This continuing cycle is amplified by the maternal immune system until a severe systemic inflammation manifests as clinical hypertension and proteinuria. The importance of circulating molecules in PE has been found clinically and experimentally; soluble fms-
like tyrosine kinase 1 is higher in PE women before clinical signs, and it induces PE-like symptoms in rodent models.\(^{22,23}\) Many uterine cell types express angiogenic and hemody
namic molecules, including human and murine uNK cells. Thus, we expect differences in the angiogenic environment of implantation sites in pregnant \( \text{Rag}^2{-}^{-}\gamma_c{-}^{-}\) mice compared with controls (decreased placental growth factor and vascular endothelial growth factor because of a lack of uNK cells and a possible relative elevation in soluble fms-like tyrosine kinase 1 because of decreased ligand availability).\(^{24,25}\) These factors were not assayed in this study, because no differences in primary outcomes (hypertension or placental insufficiency) were detected between strains.

Pregnant \( \text{Rag}^2{-}^{-}\gamma_c{-}^{-}\) females must use as-yet-unknown adaptive responses, including alterations in circulatory con
trol pathways or vasculogenesis to provide adequate placental perfusion and conceptus growth. These adaptations may involve systemic changes, including increased vasodilatory capacity, particularly of the vasculature upstream of the uterine vascular bed. This would permit decreased arterial resistance upstream of nonremodeled SAs. We postulate that, because of immune deficiency, \( \text{Rag}^2{-}^{-}\gamma_c{-}^{-}\) mice do not have damaging responses to hypoxia or oxidative stress and, thus, retain greater organ and endothelial cell function during pregnancy. Studies are in progress to validate these concepts using microultrasonography and immunohistochemical de
tection of tissue oxygenation. The as-yet-undefined,
lymphocyte-independent adaptive responses of pregnancy must be highly successful, because the sizes of the placentae of Rag2$$^{-/-}$$-$\gamma_c^{-/-}$ fetuses just before birth exceeded those of gd-matched congenic fetuses, and Rag2$$^{-/-}$$-$\gamma_c^{-/-}$ pups had no evidence of growth restriction.

Chapman et al\textsuperscript{1} monitored hemodynamic parameters of healthy cycling women who became pregnant and reported several first-trimester time points. They found a decline in gestational MAP earlier than had been appreciated previously. A nadir in MAP occurred at 8 weeks, before opening of the placental circulation.\textsuperscript{26} Arterial pressure in women remained low at 12 weeks, when the uteroplacental circulation opens, then increased but remained below or near baseline. This pattern is similar to what we describe here in mice and supports the hypothesis that, in both species, the hemodynamic changes in pregnancy are integrated temporally with specific events in placental development.\textsuperscript{27} We postulate that, in both women and mice, systemic blood pressure drops before opening of the uteroplacental circulation to enhance local hypotension and to protect fragile new developing decidual capillaries, vascular structures, and nascent fetal villi. When the placental circulation opens, the maternal vessels are mature, and the placental tissue has matured to withstand higher pressures and makes greater metabolic demand. During late gestation, when placental blood flow is high, minor variation is seen in MAP between groups (≈gd14 to term). This variation, which is not marked, may be because of differences in placental growth and metabolic demand. The concept that placental development regulates gestational MAP is further supported by the stable profiles obtained from mated females that did not conceive. This observation shows that hormones do not trigger the early drop in gestational MAP but does not assess the role of endometrial decidualization.

Others have examined the patterns of MAP during rodent pregnancy. Gestational hemodynamic data from radiotelemetric studies have been reported for normal rats\textsuperscript{28,29} and normal B6 mice,\textsuperscript{30,31} but none of these publications provide fully characterized hemodynamic profiles for normal animals, particularly using continuous radiotelemetry. Nishizawa et al\textsuperscript{32} compared SAP and DAP using indirect tail-cuff measures between syngeneically (B6$$^\text{B6}$) and allogeneically mated (B6$$^\text{BALB/c}$) mice from gd7 to gd17. Their control groups that received twice-daily placebo injections from gd6 showed drops in pressures between gd7 and gd10 followed by rebound to baseline for both groups and continued to decline for DAP in the syngeneically mated group. However, these changes were not indicated as significantly different. Zenclussen et al\textsuperscript{33} used tail-cuff recording on alternate days in a study of BALB/c mice receiving transfers of either unstimulated or type 1 cytokine-activated T cells. In nonpregnant females, neither treatment altered MAP. In pregnant females receiving nonactivated cells, MAP was reported as stable between mating and gd14. In contrast, females receiving cytokine activated (ie, nonantigen specific) T cells at gd9 and gd12 increased MAP by 93 mm Hg at gd13, and almost half of the fetuses were resorbing. Our studies reveal that the fetal loss induced in this model complicates the resolution of immune- from nonimmune-mediated effects on MAP. Our finding that 2 Rag2$$^{-/-}$$-$\gamma_c^{-/-}$ females became hypertensive before delivering small litters of dead pups implies that resorbing/dead fetuses may have a dramatic effect on MAP as early as midgestation. This finding again implies that conceptus tissue affects maternal hemodynamic control during pregnancy.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{MAP_profile.png}
\caption{Representative profile for MAP of normal mice across gestation showing a 5-phase, placental development–related pattern.}
\end{figure}
Because there is limited pathology subsequent to the deficit in SA modification and normal blood pressure regulation in Rag2\(^{-/-}\) γc\(^{-/-}\) pregnancy, a central role for the maternal immune system in the pathogenesis of PE can be postulated. Lymphocytes, through expression of a functional renin-angiotensin system, have been linked with generation of hypertension in nonpregnant rodents and humans. Human T and NK cells proliferate in vitro and modulate their cytokine production response to angiotensin II. In contrast, animal studies have addressed effects from T- and B-cell but not NK-cell deficiencies.\(^2^9\) For example, in radiotelemetric studies of nonpregnant Rag-1\(^{-/-}\) mice (T\(^{-/-}\), B\(^{-/-}\), NK\(^{+}\); sex not stated), Guzik et al\(^3^5\) found that absence of T cells blunted the ability to induce hypertension by challenge with angiotensin II or dietary salt. Adoptive transfer of purified T cells but not B cells restored the angiotensin II response. The T cells’ role was attributed to the production of tumor necrosis factor-α. The recent development of a genetically defined T\(^{+}\), B\(^{+}\), NK\(^{-}\) mouse will now permit similar studies addressing the functional roles of the renin-angiotensin system in NK cells.\(^3^6\) However, it is predicted from our experiments that immune responses to placental signals are critical amplifiers of pathogenic processes leading to pregnancy complications.

**Perspectives**

Our overall definition of the gestational pattern of MAP in normal mice and its linkage to stages of placental development is the major contribution of this study. By focusing on changes in human gestational MAP at times of specific developmental events (eg, presence or absence of change in MAP at the time of opening of the placental circulation or days or rate of gain to achieve prepregnancy baseline MAP), it may be possible to more specifically identify the time at which developmental programming errors onset, triggering clinically significant complications. This temporal characterization should also aid in identification of causation. We also provide clear evidence that normal SA remodeling is not essential for normal pregnancy outcomes in mice. In addition, we have provided the pregnant Rag2\(^{-/-}\) γc\(^{-/-}\) mouse as a hemodynamically, well-defined animal platform lacking SA remodeling but having normal placental growth. This makes it an ideal animal for subsequent reconstruction studies in which specific molecules, such as tumor necrosis factor-α, soluble fms-like tyrosine kinase-1, placental growth factor, or the angiotensin II type 1 agonist antibodies, molecules that are postulated to have major roles in pregnancy complications, can be independently and incrementally assessed for the induction of hypertension, placental pathology, and fetal loss.\(^2^9\) This platform also potentially permits assessment of highly targeted therapeutic approaches.

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**Disclosures**

None.

**References**


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