Impact of Immune Deficiency on Remodeling of Maternal Resistance Vasculature 4 Weeks Postpartum in Mice

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Abstract

Pregnancy manifests changes in the vascular and immune systems that persist postpartum (PP), have important implications for future pregnancies, and may modify responses to cardiovascular stress in late life. The association between immune and vascular function and the generation or progression of cardiovascular disease beg the question of whether altered immunity modifies pregnancy-induced changes in the vasculature. Our objective was to compare changes in the function and remodeling of systemic resistance vessels 4 weeks PP in normal C57BL/6 (B6), and immunodeficient mice recombinase I-deficient/B6 (*Rag1*^{-/-}). Immune deficiency did not change the responsiveness to acetylcholine (ACh) and phenylephrine at baseline but decreased arterial distensibility and increased stiffness PP. Adoptive transfer of CD8 T cells into *Rag1*^{-/-} mice decreased the response to ACh while increasing distensibility and wall thickness. When compared to PP *Rag1*^{-/-}, vessels from PP CD4-deficient mice, which have B cells and CD8 T cells, but no CD4 cells, show increased distensibility and decreased responsiveness to ACh in a pattern similar to that seen in *Rag1*^{-/-} given CD8 T cells prior to mating. These studies suggest a key role for T cell, particularly CD8 T cell, associated factors in the PP remodeling of maternal resistance vessels.

Keywords

T cells, B cells, vascular remodeling, pregnancy, postpartum, mice

Introduction

Normal pregnancy is associated with significant maternal cardiovascular adaptation that is critical for fetal survival and development. In the course of pregnancy, cardiac output is increased¹ in addition to blood volume expansion.^{2,3} Both systemic and uterine vascular resistances are decreased,^{4,5} due to vasodilation in mesenteric⁶ and uterine circulations,⁶⁻⁸ and this enhances blood flow to the uterus. In addition, pregnancy induces changes in structural parameters such as distensibility in the uterine vessels⁵ and stiffness in the systemic vasculature,⁹⁻¹¹ among other important modifications of vascular response.

Much less is known about maternal cardiovascular changes in the postpartum (PP) period. However, some degree of pregnancy-induced vascular adaptation continues PP^{9,12-14} and includes increased cardiac output, decreased peripheral vascular resistance,¹³ and changes in vascular compliance⁹ and is regulated by molecules such as nitric oxide (NO)¹⁵ and by changes in cardiac structure and function.^{16,17} Moreover, PP systemic vasculature may exhibit new remodeling, such as outward remodeling of mesenteric resistance arteries,¹² which may result in the redistribution of the cardiac output

systemically as blood flow to the uterus decreases to approximate the prepregnancy condition.

With regard to the immune system, pregnancy is a state of significant changes, including the expansion of regulatory T cells (TREGs),^{18,19} CD8 T cells specific for paternal²⁰ and fetal antigens, and enhanced turnover in other leukocyte populations.²¹⁻²⁴ We have studied T-cell homeostasis in mice and observed changes in the maternal immune system that persist PP.^{21-23,25-27} There may be accumulation of TREGs in PP that may protect subsequent pregnancies.^{28,29} Abnormal pregnancy, including preeclampsia³⁰⁻³⁵ and preterm birth,³⁶ is associated with immune system dysregulation. Moreover, T-cell subset

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manipulation has revealed and modified cardiovascular abnormalities during normal rodent pregnancy³⁷ and in rodent models of pregnancy-associated hypertension.^{33,34} However, the immunology of the PP window and its potential contribution to PP maternal cardiovascular parameters has not been fully examined. Our objective therefore was to examine the role of immune deficiency in the PP structural and functional characteristics observed in the systemic resistance vessels. We chose to examine normal mice and those with genetic absence of T cells and B cells but which have natural killer (NK) T cells (*Rag1*^{-/-} mice³⁸), in order to focus on the critical role of the adaptive immunity.

The CD8 T cells can respond to fetal antigens during pregnancy³⁹⁻⁴¹ and their phenotype is influenced by multiparity.⁴² In addition, CD8 T cells exist that are specific for vascular endothelial growth factor,⁴³ and other vascular components⁴⁴ play a role in reactivity to vasoconstrictors or vasodilators⁴⁵ and influence the development of hypertension and cardiovascular disease.⁴⁶⁻⁴⁹ Because of these characteristics, we examined the effects of CD8 T-cell reconstitution in *Rag1*^{-/-} mice. In light of the potential role played by CD4 TREGs in the immune homeostasis of pregnancy^{18,50} and in the modification of cardiovascular disease,^{46,51-54} we also examined mice with genetic deficiency in CD4 T cells.⁵⁵

We chose mesenteric vessels because of their role in the regulation of systemic blood pressure.^{6,56} We observed overall that while the characteristics of vessels from naive normal and immunodeficient mice were similar, pregnancy and the PP period differentially influenced resistance vasculature in normal and immunodeficient mice. Further, we found that this differential influence was ameliorated by CD8 T cells in the absence of CD4 T cells.

Materials and Methods

Mice and Tissues

The C57BL/6 (B6), recombinase 1-deficient mice on a B6 background (*Rag1*^{-/-}),⁴¹ and CD4-deficient⁵⁷ females 3 to 5 months of age were either left virginal (never pregnant [NP]) or mated to same-strain males. Four weeks after delivery of a litter, or approximately 3 to 7 days after the weaning of pups, the mice that became pregnant (PP) and naive age-matched mice were euthanized by cervical dislocation after CO₂ sedation. The mesenteric vascular arcade was quickly removed and placed in preaerated (5% O₂, 10% CO₂, and 85% N₂) physiologic salt solution ([PSS] 119 mM NaCl, 4.7 mM KCl, 24.0 mM NaHCO₃, 1.2 mM KH₂PO₄, 1.6 mM CaCl₂, 1.2 mM MgSO₄, 0.023 mM EDTA, and 11.0 mM glucose, pH = 7.4, all chemicals were purchased from Fisher Scientific, Atlanta, GA). Second-order mesenteric arteries were identified, removed, and cleaned of perivascular tissues. Vessels were cannulated from both ends in a pressure arteriograph placed on the stage of an inverted microscope with an attached video camera. Arteries were pressurized at 10 mm Hg using a Servo pressure system (Living Systems Instrumentation, Burlington,

Vermont) and continuously superfused at 3 mL/min with aerated PSS at 37°C and pH 7.4 for 1-hour equilibration period before starting an experimental protocol.

Response to Vasodilators or Vasoconstrictors

After the equilibration period, the intraluminal pressure was elevated from 10 to 50 mm Hg. To test the endothelial function, the artery was precontracted with phenylephrine (PE; Sigma, St Louis, Missouri) to 50% to 60% of the initial diameter, and ACh (0.01–10 µM; Sigma) was applied in a cumulative fashion. In another artery, PE was tested in increasing concentrations (0.01–30 µM). Changes in the arterial diameter were monitored using the IonOptix program (version 6.4). Dose–response curves for ACh-induced vasodilatation and PE-induced vasoconstriction were constructed using the Sigma-Plot program (version 12.5).

Mechanical Behavior of Arteries

Pressurized at 3 mm Hg, arteries were superfused for 10 minutes with PSS containing 20 µM diltiazem (Sigma; prepared as a 10-mM stock solution in deionized water and kept refrigerated until use) and 50 µM papaverine (Sigma; dissolved in deionized water on the day of the experiment) to inhibit vascular contractility and to allow for maximal arterial dilation at each pressure. Inner (lumen, D_{in}) and outer (D_{out}) arterial diameters were measured after stepwise elevation in intraluminal pressure from 3 to 120 mm Hg. A 3 mm Hg was the minimal pressure for unstretched vessel that prevented vessel collapse. Diameters were measured from the images of pressurized arteries on the monitor screen after stabilization of the lumen diameter at each specific level of pressure (typically, 2–3 minutes for each pressure step). Arterial wall thickness (t) was calculated as follows: $t = (D_{out} - D_{in})/2$. Arterial distensibility was defined as an increment in lumen diameter in response to pressure elevation from 3 to 120 mm Hg. The increments in lumen diameter are expressed as the percentage of a unstretched vessel diameter at 3 mm Hg. For calculation of circumferential wall stress, intraluminal pressure was converted from mm Hg to N/m² (1 mm Hg = 1.334×10^2 N/m²). Circumferential stress (σ) was calculated using the following equation: $\sigma = P \times (D_{in}/2) \times t$. Circumferential strain (ϵ) was calculated according to the following equation: $\epsilon = (D_{in} - D_{in3})/D_{in3}$, where D_{in3} represents the lumen arterial diameter at the lowest intraluminal pressure (3 mm Hg). The stress versus strain curve was fitted to an exponential function using Sigma-Plot program and the rate constant of this function is reported as the stiffness coefficient.⁵⁸

Reconstitution of *Rag1*^{-/-} Mice With CD8 T Cells

Rag1^{-/-} females aged approximately 2 months were given 2 to 10 million thymocytes or spleen CD8 T cells isolated from aged-matched B6 females by negative or positive magnetic cell sorting (Miltenyi Biotec, Auburn, California). Mice subjected

Figure 1. Response to acetylcholine (ACh) in mesenteric arteries from never pregnant (NP) and postpartum (PP) C57BL/6 (B6) and *Rag1*^{-/-} mice. Vessels (n = number of vessels/number of mice) were mounted and pressurized to 50 mm Hg and precontracted with phenylephrine to 50% to 60% of the initial diameter before stepwise exposure to ACh followed by exposure to 20 μmol/L diltiazem and 50 μmol/L papaverine to obtain maximal dilation. Y-axis, percentage of maximal dilation. X-axis, concentration of ACh in μM. *Significant difference at the level of *P* < .05 by repeated-measures analysis of variance (ANOVA). Black circles, vessels from never pregnant (NP) C57BL/6 mice. Black triangles, vessels from postpartum (PP) C57BL/6 mice. White circles, vessels from NP *Rag1*^{-/-} mice. White triangles, vessels from PP *Rag1*^{-/-} mice. Gray arrow points in the direction of less responsiveness to ACh. A, Comparison of NP and PP C57BL/6 mice. B, comparison of NP C57BL/6 to NP *Rag1*^{-/-} mice. C, Comparison of NP to PP *Rag1*^{-/-} mice.

to reconstitution were bled periodically and the blood samples were treated with antibodies to CD8-α (clone 53-6.7; BD BioSciences, San Jose, California) and CD4 (clone GK1.5; BD BioSciences) and subjected to flow cytometry (LSRII; BD BioSciences) to confirm the presence of CD8 T cells systemically and to delineate the kinetics of the presence of CD8 T cells. At euthanization, mice were also tested for the presence of CD8 T cells in the peripheral blood and spleen. Herein, we report mice that were reconstituted only with CD8 T cells and found that their presence persisted into PP (data not shown).

Statistics

The number of mice and vessels chosen was to detect an effect seen in the previous work.⁵⁸ Comparison of structural parameters and gene expression from groups of vessels was performed with *t* test or Mann-Whitney test. Functional studies were analyzed and vessels from the 2 groups of mice were compared by 2-way repeated-measures analysis of variance. Summary variables, for example, stiffness coefficient, were compared by *t* test. Hypothesis testing used a probability of *P* < .05 as the cutoff for significance. We used Sigma-Plot versions 11 and 12.5 (Systat software Inc, San Jose, California)

and GraphPad Prism (version 6; La Jolla, California) to analyze data and perform statistical analysis.

Results

Vasodilator and Vasoconstrictor Reactivity

Vasodilator reactivity to ACh of PP B6 mesenteric vessels was not significantly different from that of vessels from NP B6 mice (Figure 1A). While vessels from NP *Rag1*^{-/-} mice were less responsive to ACh than those from B6 (Figure 1B), in the PP period, *Rag1*^{-/-} vessels were slightly more responsive as compared to the NP (Figure 1C). Thus, the PP period likely results in opposing tendencies related to the ACh-induced dilator response in vessels from normal and immunodeficient mice.

An important element in the regulation of blood vessels is the response to vasoconstrictor agents. For these studies we chose to examine the mesenteric vessel response to PE.⁵⁸ By 4 weeks PP, the vessel response to this agent was similar to NP in both B6 (Figure 2A) and *Rag1*^{-/-} mice (Figure 2C). In addition, we did not observe inherent difference in vessels from NP B6 versus *Rag1*^{-/-} mice, leaving open the possibility for transient differences in vessel responsiveness over time.

Figure 2. Response to phenylephrine is similar in mesenteric arteries from never pregnant (NP) and postpartum (PP) B6 and *Rag1*^{-/-} mice. Vessels (n = number of vessels/number of mice) were mounted and pressurized to 50 mm Hg before stepwise exposure to phenylephrine. Y-axis, percentage of maximum constriction. X-axis, concentration of phenylephrine in assay medium in μM . Symbols and comparisons in panels A, B, and C are as in Figure 1 above.

Immune Deficiency Modifies Structural Changes Observed in Mesenteric Vessels 4 Weeks PP

We next determined the effect of PP and the immune system on mechanical behavior of systemic resistance vessels in mice. The passive arterial lumen diameters and arterial wall thicknesses were measured from vessels pressurized at 50 mm Hg—a pressure at the lower range of mean arterial pressure in normal mice.⁵⁹ There was a trend toward increase in passive lumen diameters and significantly increased wall thickness of PP vessels from B6 mice as compared to vessels from age-matched, NP mice (Figure 3A). These parameters were similar in NP B6 and immunodeficient *Rag1*^{-/-} (Figure 3B). However, PP did not affect the change in the vessels from PP *Rag1*^{-/-} mice as compared to NP *Rag1*^{-/-} mice (Figure 3C).

We next characterized the effects of PP and immune deficiency on distensibility of mesenteric resistance vasculature. The increments in passive lumen diameter in percentage from lumen diameter of unstretched vessels (at 3 mm Hg) were plotted as a function of intraluminal pressure. The PP period in B6 mice was associated with an increase in distensibility of mesenteric arteries as compared to the vessels from NP B6 mice (Figure 4A). The distensibility of vessels from B6 and *Rag1*^{-/-} NP mice was not significantly different (Figure 4B). In contrast to the vessels from B6 mice, PP *Rag1*^{-/-} vessels exhibited reduced distensibility as compared to those from NP *Rag1*^{-/-} mice (Figure 4C). Finally, distensibility of mesenteric arteries from PP *Rag1*^{-/-} mice was significantly reduced compared to that of vessels from PP B6 mice (Figure 4D).

To characterize the effects of immune deficiency on arterial wall stiffness, stress, and strain, we calculated the circumferential stress-strain relationship for mesenteric arteries using Sigma-Plot. There were no significant difference in stiffness coefficient of arteries from B6 NP versus B6 PP mice (Figure 5A). However, the PP period was associated with significant increase in wall stiffness of arteries from *Rag1* compared to that of B6 mice (Figure 5B). Thus, immune deficiency leads to altered mechanical behavior in resistance mesenteric vasculature during the PP period.

The CD8 T Cells May Play a Role in PP Mesenteric Structure and Function

CD8 T cells are crucial to certain antiviral^{60,61} and antitumor responses and antitransplant response.⁶² Activated CD8 T cells contribute to the cytotoxic responses that can clear circulating fetal antigen³⁹ and support antiviral immunity during pregnancy.²⁵ CD8 T cells can also respond to vascular components^{43,44} and may play a role in reactivity to vasoconstrictors or vasodilators⁴⁵ and in the development of hypertension and cardiovascular disease.⁴⁶⁻⁴⁹ Based on these observations, we examined the effects of CD8 T-cell reconstitution in *Rag1*^{-/-} mice prior to pregnancy and in the PP period. In the 3- to 4-month-old NP mice, the exposure to CD8 T cells did not affect vasodilator reactivity to ACh (Figure 6A), passive lumen diameters, and wall thickness of mesenteric vessels (Figure 6B). However, the presence of CD8 T cells was associated with increased vessel distensibility (Figure 6C) and reduced arterial wall stiffness (Figure 6D).

Figure 3. Lumen diameter and wall thickness in mesenteric arteries from never pregnant (NP) and postpartum (PP) B6 and *Rag1*^{-/-} mice. Vessels (n = number of vessels/number of mice) were mounted and treated with 20 $\mu\text{mol/L}$ diltiazem and 50 $\mu\text{mol/L}$ papaverine before stepwise pressurization. Left panels, lumen diameter in μm . Right panels, wall thickness in μm . Black bars, vessels from NP C57BL/6 mice. White bars, vessels from NP *Rag1*^{-/-} mice. Hatched bars, vessels from PP mice. *Significant difference at $P < .05$ by Student *t* test.

Figure 4. Distensibility of mesenteric arteries from never pregnant (NP) and postpartum (PP) B6 and *Rag1*^{-/-} mice. Vessels (n = number of vessels/number of mice) were harvested and treated as in Figure 1. The change in vessel diameter as a function of pressure was plotted and compared. Y-axis, percentage of lumen diameter observed at 3 mmHg. X-axis, pressure in mmHg. Symbols and comparisons in panels A, B, and C are as in Figure 2 above. D, Comparison of vessels from PP B6 and PP *Rag1*^{-/-}. *Significant difference at the level of $P < .05$ by repeated-measures analysis of variance (ANOVA).

Figure 5. Stiffness coefficient in postpartum (PP) B6 and *Rag1*^{-/-} mice. The stiffness coefficient (β) was calculated as described in the methods, incorporating the structural parameters measured. Bars are as in Figure 3 above. Left panels, circumferential stress-strain curves. Right panels, calculated stiffness coefficient. A, Comparison vessels from never pregnant (NP) to PP B6 mice. B, Comparison of vessels from PP B6 and PP *Rag1*^{-/-} mice. *Statistically significant difference by Student t test.

Prepregnancy establishment of CD8 T cells in *Rag1*^{-/-} mice supported both functional structural and changes observed PP (Figure 7). Postpartum wall thickness was increased, but vessel lumen diameter was unchanged in vessels from *Rag1*^{-/-} mice receiving CD8 T cells as opposed to those vessels from control *Rag1*^{-/-} mice (Figure 7B). Further, distensibility was increased (Figure 7C) and wall stiffness was reduced (Figure 7D) PP in vessels from treated mice. Finally, ACh-induced vasodilation in the PP period of CD8-exposed mice was markedly decreased as compared to controls (Figure 7A) and approached that of vessels from NP *Rag1*^{-/-} mice (Figure 1B).

The *Rag1*^{-/-} mice injected with CD8 T cells have neither CD4 T cells nor B cells. The CD4 TREGs^{46,52} and B cells⁶³ have been observed to mediate vascular physiology and pathology. To begin to determine the potential role played by the interaction between CD8 T cells and B cells in the absence of CD4 T cells in PP vascular physiology, and to address the relatively low systemic levels of CD8 T cells in the adoptive

transfer experiments, we began the analysis of vessels from PP CD4-deficient mice⁵⁷ (Figure 8). We observed that, as in *Rag1*^{-/-} mice injected with CD8 T cells, the PP period resulted in vessels that were markedly less responsive to ACh (Figure 8A) but more distensible in response to pressure elevation (Figure 8C) as compared to vessels from untreated PP *Rag1*^{-/-} mice. In addition, arterial wall stiffness was significantly reduced in CD4-deficient versus *Rag1*^{-/-} PP mice (Figure 8D). Thus, PP vessels from CD4-deficient mice behaved similarly to vessels from *Rag1*^{-/-} mice that received CD8 T cells prior to pregnancy.

Discussion

Our observations suggest that (1) the PP is associated with a persistent increase in arterial distensibility but not with significant alterations in mesenteric artery reactivity to ACh and PE as compared to NP, (2) immunodeficiency abrogates the observed arterial structural remodeling associated with PP,

Figure 6. Effect of adoptive transfer of CD8 T cells on characteristics of mesenteric arteries from never pregnant (NP) mice. Female $Rag1^{-/-}$ mice received 2 million CD8 T cells at 2 months of age and mesenteric vessels from adoptively transferred or nonrecipient age-matched controls were examined 1 to 3 months later. A, Response to acetylcholine (ACh). B, Left panel, lumen diameter. Right panel, lumen wall thickness. C, Distensibility. D, Stiffness coefficient. White bars or symbols, vessels from nonrecipient controls. Gray-filled bars or symbols, vessels from recipients of CD8 T cells. A and C, *Significant difference at the level of $P < .05$ by repeated-measures analysis of variance (ANOVA). B and D, *Significant difference at the level of $P < .05$ by Student t test.

(3) adoptive transfer of CD8 cells prior to mating results in restoration of PP-associated remodeling of resistance arteries, and (4) vessels from PP CD4-deficient mice show an increased distensibility and decreased responsiveness to ACh in a pattern similar to that seen in $Rag1^{-/-}$ given CD8 T cells. Normal pregnancy is associated with significant maternal cardiovascular changes that include altered vasodilator reactivity and compliance of maternal uterine and systemic vasculature in animal models^{6,64} and in humans.^{10,65,66} Although existing evidence suggests that pregnancy-driven functional vascular changes are resolved by the PP period, structural changes persist in the PP vasculature of animals¹² and women.⁹ Our findings correlate well with these data. Taken together, these observations suggest that persistent reduction in systemic vascular resistance in the PP period is most likely induced by long-term changes in vascular remodeling rather than in vascular function.

These studies may have implications for the study of long-term cardiovascular health. Normal pregnancies are generally associated with very-low-long-term cardiovascular risks, while complicated pregnancies, including preeclampsia,⁶⁷⁻⁶⁹ preterm birth,⁷⁰⁻⁷² gestational diabetes,⁷³ and high multiparity,⁷⁴ are associated with increased long-term risk for cardiovascular disease. The examination herein raises the possibility that

analysis of PP structural vascular properties along with innate and adaptive immune parameters will inform refined studies in the human PP. These, in turn, will ultimately serve to suggest biomarkers of future cardiovascular risk in women with adverse pregnancy outcomes.

These studies have limitations. Our observations herein were focused on PP after a single pregnancy. However, we would expect that our findings would have implications for future pregnancies, particularly if those pregnancies undergo inflammatory stress.^{36,75} Although we have presented firm data as to the findings and a “proof of principle,” that the immune system matters in the PP vascular remodeling, we as yet do not have a specific mechanism for the effect on the major vascular components. This will have to be addressed in future in vivo and in vitro studies.

The exact causes and mechanisms underlying resistance artery remodeling in pregnancy and PP period remain poorly defined. While growth and remodeling of the maternal uterine vasculature is in part induced by a marked increase in uterine blood flow, it is not clear how this remodeling is reversed PP.⁵ Much less is known about structural changes in the PP systemic vasculature. In mice, an increase in the lumen diameters of mesenteric arteries was observed only in the PP period, and this was hypothesized to be related to elevated mesenteric

Figure 7. Effect of adoptive transfer of CD8 T cells on characteristics of mesenteric arteries from postpartum (PP) mice. Female $Rag1^{-/-}$ received 2 million CD8 T cells at 2 months of age and then mated at 3 months of age. Four weeks after delivery of 1 litter, mesenteric vessels from adoptively transferred or nonrecipient controls were examined. White hatched bars or symbols, vessels from nonrecipient controls. Gray-filled hatched bars or symbols, vessels from recipients of CD8 T cells. A, B, C, and D as in Figure 6.

Figure 8. Characteristics of mesenteric arteries from postpartum (PP) CD4-deficient mice. $Cd4^{-/-}$ mice were mated and allowed to litter. Four weeks after delivery, mesenteric vessels were analyzed. Shown are data from $Cd4^{-/-}$ mice (black diamonds or cross-hatched gray bars) compared to previous data from PP $Rag1^{-/-}$ mice (white triangles or hatched bars). A, B, C, and D are as in Figure 6.

blood flow.¹² In our study, we found a residual increased distensibility in the maternal mesenteric arteries of normal mice at 4 weeks PP but not in vessels from immunodeficient mice. Arterial compliance is controlled by multiple mechanisms, including arterial wall collagen–elastin composition, smooth muscle cell plasticity, and may involve molecules such as relaxin,⁷⁶ matrix metalloproteinases,⁶⁴ sex steroids,⁷⁷ fibulin,⁷⁸ angiotensin, and NO, among others. The mechanisms contributing to persistent change in systemic arterial compliance PP require further investigation.

The premise for these studies is that evidence for systemic vascular remodeling is present during PP and that the immune system may play a role. In normal B6 mice, mesenteric vessels exhibit increased responsiveness to pressure (distensibility) but slightly decreased response to ACh in the PP as compared to vessels from NP mice. In contrast, mice deficient in T cells and B cells, but not NK cells, exhibit opposing changes including decreased distensibility and slightly increased response to the vasodilator ACh. In *Rag1*^{−/−} mice, these findings are in part reversed by prepregnancy administration of CD8 T cells. For example, the stiffness coefficient for PP CD8-injected *Rag1*^{−/−} mice (Figure 7D) is the same as in PP B6 vessels (Figure 5B right).

The absence of B cells and CD4 T cells in *Rag1*^{−/−} mice given CD8 cells tends to “overshoot” the PP effect on ACh responsiveness in B6 mice, producing even less responsiveness to ACh (Figure 7A). This effect of CD8 T cells in some respect is dissimilar to the protective effect of adoptive transfer of CD8 T cells against vascular damage in older male mice⁷⁹ and may be related to sexual dimorphism in the effect of immune cells on vascular function.^{59,80} Addition of B cells to the system (eg, *Cd4*^{−/−} mice, Figure 8) produces a similar overshoot in decreased ACh responsiveness (Figure 8A) and produces even greater distensibility (Figure 8B). This is somewhat consistent with data suggesting that adoptive transfer of B cells in *Rag1*^{−/−} mice is protective against vascular damage in older male mice.⁶³ These studies extend the findings in *Rag1*^{−/−} mice during pregnancy³⁷ and suggest that study of PP may reveal interactions between the immune and vascular system that continue to evolve over time.

The CD8 T cells transferred to *Rag1*^{−/−} mice before mating were a mixture of naive and memory T cells and likely underwent homeostatic proliferation⁸¹ before mating. This may have either changed their phenotype or selected a specialized subset of CD8 T cells not normally present in pregnant B6 mice. However, we have shown that pregnancy in normal mice supports turnover in the CD8 T-cell population,^{21,22} and it is likely that such homeostatic turnover occurs similarly, but perhaps to a slightly greater extent in CD8 T cells transferred to *Rag1*^{−/−} mice and in CD8 T cells from normal B6 mice under the influence of pregnancy and PP.

There is a sexual dimorphism in the response to angiotensin II in *Rag1*^{−/−} as compared to B6 mice,⁸⁰ and this difference has been related to a function specific to male T cells. While pregnancy in B6 mice does subject the female to the presence of circulating male fetal cells,⁴⁰ it is not clear that enough male

fetal T-cell trafficking occurs to the mother to contribute to the PP vascular response. This could be tested in future by the analysis of the vessels of *Rag1*^{−/−} females mated to normal B6 males.

The model we suggest is that both the immune system and the vascular system undergo changes during pregnancy that subsequently play out in unique adaptation in the PP. Such changes may result from the competition between cells supporting the response of each system and may be related to critical cytokines and growth factors. For example, interleukin 15 is tightly regulated⁸² and important for vascular remodeling at the maternal fetal interface⁸³ for CD8 T-cell homeostasis^{84,85} and for tissue-resident T-cell development.⁸⁶

Based on these findings herein, and others,^{37,50} we speculate that unique interaction between immune and vascular systems is set by the experience of pregnancy and placenta-related factors⁸⁷ and continues in PP. This in turn influences subsequent susceptibility to cardiovascular stress. This will need to be studied in carefully designed experiments in humans and animal models.

Declaration of Conflicting Interests

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