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EDITORIAL

Don't Break the Axis: Placental Inflammation Leads to Congenital Heart Disease

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The placenta is a transient organ formed during pregnancy that acts as a barrier to the passage of pathogens and cells and as an exchange interface, ensuring supply of oxygen and nutrients to the fetus and the elimination of waste products derived from fetal blood. Human placentation involves the remodeling of the maternal spiral arteries by invading trophoblast cells to generate a low-resistance maternal uterine circulation.[1] Abnormal placental blood vessel remodeling can produce high-resistance maternal uterine circulation, leading to preeclampsia, a pregnancy complication that disrupts the supply of oxygen and nutrients to the fetus and typically features high maternal blood pressure and proteinuria. Preeclampsia affects between 5% and 7% of pregnancies and is responsible for 70 000 maternal and 500 000 fetal deaths per year worldwide.[2] Typical preeclampsia risk factors are first-time pregnancy, preeclampsia in previous pregnancies or a family history of the condition, hypertension, kidney disease, diabetes, autoimmune disease, and obesity.[3] Preeclampsia can occur early (preterm preeclampsia; <34 weeks of gestation) or late (term preeclampsia; after 34 weeks) in pregnancy. Management of preeclampsia includes preconception counseling, perinatal blood pressure control, prenatal aspirin therapy in high-risk women,[4] betamethasone therapy <34 weeks, parenteral magnesium sulfate, and careful monitoring of postpartum blood pressure.[3] Definitive treatment of preeclampsia requires either preterm delivery, with its attendant risks, or careful management of the condition until delivery is feasible. Severe preeclampsia requires hospitalization and is treated with antihypertensive drugs to lower blood pressure and anticonvulsant medication to prevent seizures. In addition to severe consequences for the mother, unattended preeclampsia increases the risk of congenital heart defects (CHDs) in the baby.[5] The heart is the first organ to develop and function in the human embryo, beginning as a linear pumping tube that organizes progressively into the complex 4-chamber mature organ. CHDs encompass a range of structural birth defects that impair normal heart function and affect ≈1% of live births worldwide.[6] Heart and placenta development are interdependent,[7,8] attributable to a proposed placenta–heart communication axis that may reflect a shared biochemical environment.[9]

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In this issue of *Circulation*, Ward et al[10] show that experimental neutrophil-driven placental inflammation (NDPI) in mice leads to abnormal placental development and loss of barrier function. This allows maternal placental inflammatory monocytes to migrate to the embryonic heart, skewing the composition of the resident cardiac macrophage population and altering ventricular structure. Cardiac alterations manifest postnatally and involve both tissue organization and function. Experimental attenuation of placental inflammation prevents fetal cardiac defects and allows normal postnatal cardiac function (Figure). Women with preeclampsia have an activated neutrophil phenotype[11] and an elevated risk of having offspring with CHDs.[12] Thus, the authors suggest that neutrophil phenotyping may be an early test to monitor for potential CHD in fetuses. Further experimental studies will demonstrate whether targeting early placental inflammation with

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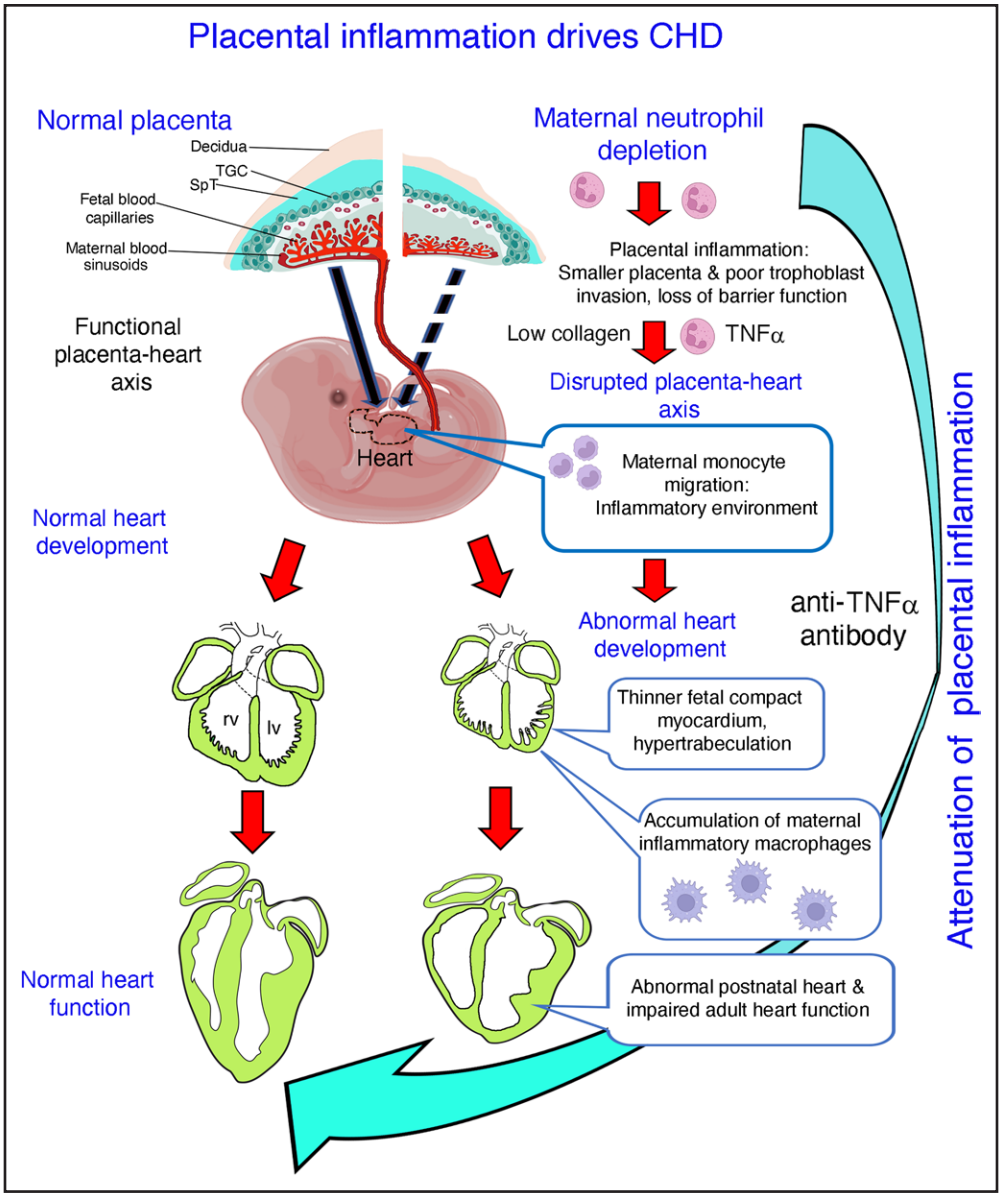

Figure. Neutrophil-driven placental inflammation in pregnant mice leads to a preeclampsia-like placental phenotype that disrupts cardiac development.
Left, Normal placentation is essential for the establishment of a functional placental–heart axis that allows normal heart development and adult heart function. **Right**, maternal neutrophil depletion leads to placental inflammation, low collagen expression, and poor trophoblast cell invasion. Placental neutrophils express high amounts of inflammatory tumor necrosis factor–α (TNFα). Loss of placental barrier function allows migration of maternal CCR2-positive monocytes to the heart. The proinflammatory environment in the embryonic heart interferes with its normal development, indicated by hypertrabeculation and reduced proliferation of endothelial and cardiomyocyte cells. Fetal heart abnormalities negatively affect adult heart function. Thus, placental inflammation may cause congenital heart defects (CHDs). Blue arrow: A potential therapeutic approach would be to attenuate neutrophil-driven placental inflammation with an anti-TNFα antibody infusion, which is sufficient to rescue cardiac development and function both in experimental neonates and in adults. SpT indicates spongiotrophoblast; and TGC, trophoblast giant cell.

anti–tumor necrosis factor–α (TNFα) antibodies may help restore normal placental and cardiac development and function, eventually offering a real alternative to current invasive interventions. The study presents solid data on the structural and inflammatory phenotype of NDPI placentas, but more experiments are needed to establish these mice as a preclinical model of preeclampsia (see below). Further experiments will be needed to define the fetal and postnatal cardiac phenotypes.

A Mouse Model of Placental Inflammation That Recapitulates Preeclampsia Features and Impairs Cardiac Development

The authors show that their established model of maternal neutrophil depletion during murine pregnancy[13] results in smaller placentas, characterized by poor trophoblast invasion, inflammation, and loss of barrier function, all typical features of preeclampsia.

They also show that maternal monocytes migrate to the fetal heart, disrupting cardiac development and postnatal cardiac function. To establish NDPI as a preeclampsia model, additional experiments should examine whether NDPI pregnancies include the typical preeclampsia features of increased maternal blood pressure and proteinuria.

Are There Differences in Gene Expression Between Control and NDPI Placentas?

The authors generated transcriptional profiles of the NDPI placenta and controls. They focused on collagen genes because they are crucial for placental tissue integrity,[14] and 17 of these were downregulated. Immunofluorescence revealed lower collagen I expression in the NDPI decidua, as well as collagen IV expression in both the decidua and labyrinth. Collagen IV is required for trophoblast invasion, and its reduced expression may explain the poor trophoblast invasion in NDPI. The authors then show that collagen I expression was significantly attenuated in human CHD placentas compared with control and preeclamptic placentas, whereas collagen IV was reduced in both preeclamptic and CHD placentas. The authors conclude that NDPI disrupts placental structure. Whereas the structural defects of NDPI placentas are well supported, deeper investigation is warranted to examine their vascular structure (ie, spiral arteries) and the status of the different placental trophoblast cell types.

How Does NDPI Affect Heart Development?

Abnormal placental development impairs cardiac development,[7–9] and E14.5 NDPI hearts had a thin left ventricle compact myocardium and showed defective endocardial differentiation. Moreover, these defects were accompanied by defective smooth muscle cell differentiation. Three-dimensional episcopic imaging revealed thinner and longer trabeculae in NDPI hearts, suggesting a hypertrabeculation phenotype. This was accompanied by reduced endothelial cell proliferation, attenuated expression of angiogenic regulators, and lower cardiomyocyte proliferation, indicating impaired heart development and embryonic cardiac vascularization. How NDPI causes CHDs could be helpfully investigated through detailed heart-lineage–specific developmental marker analysis using single-cell RNA sequencing, in situ hybridization, and immunohistochemistry.

How Do Maternal Proinflammatory Leukocytes Accumulate in Embryonic Hearts?

The authors found a 4-fold increase in the number of maternal proinflammatory leukocytes in NDPI embryonic hearts, a finding confirmed by adoptive transfer of GFP^+ splenic leukocytes from age-matched females into the maternal circulation and validated by the increased leakage of fluorescein isothiocyanate–dextran in NDPI placentas. These results suggest that exacerbated placental inflammation promotes a breakdown in placental tissue barrier function, allowing maternal proinflammatory leukocytes to enter the embryonic heart, where they interfere with the normal development of this organ.

What Is the Leukocyte Composition in the Embryonic Heart?

Analyses by scRNA sequencing of $CD45^+$ leukocytes isolated from E14.5 NDPI hearts revealed that fetal resident macrophages expressed genes typical of yolk sac–derived macrophages, whereas maternal leukocytes expressed genes associated with adult macrophages. Additional studies on the functional consequences of these macrophage populations in the adult heart would be of interest.

Do Cardiac Developmental Alterations Persist After Birth and Affect Cardiac Function?

Postnatal day 5 (P5) mice from NDPI pregnancies were smaller and had a higher heart:body weight ratio than controls. These hearts also showed hypertrabeculation and a less compact endocardial structure. Ultrasound of NDPI offspring at P28 revealed significantly reduced cardiac output, stroke volume, ejection fraction, and fractional shortening. The phenotype of $CD45^+$ leukocytes in P28 hearts was not altered, but the proportion of $F4/80^+Ly6C^+$ macrophages was increased in P28 hearts from NDPI offspring. Further detailed analysis by scRNA sequencing of P5 and P28 hearts derived from NDPI pregnancies would help determine the molecular basis of these structural and functional defects.

Can the Attenuation of Placental Inflammation be Therapeutically Useful to Rescue Cardiac Development and Function?

The authors reduced local inflammation by injecting pregnant NDPI mice with a neutralizing antibody against TNFα. Blocking TNFα reduced placental TNFα levels and rescued placental tissue organization, enabled deeper trophoblast invasion, and restored placenta collagen expression. In addition, placentas in NDPI+ anti-TNFα pregnancies had reduced numbers of inflammatory monocytes and activated placenta neutrophils, which expressed lower levels of TNF. The E14.5 NDPI+ anti-TNFα heart appeared normal, and endothelial cells and cardiomyocytes proliferated normally. At P28, the hearts of NDPI+ anti-TNFα offspring showed normal function compared with NDPI offspring, and fewer $F4/80^+Ly6C^+$ macrophages than NDPI offspring, suggesting a quiescent, anti-inflammatory phenotype. These results

suggested that anti-TNFα injection during pregnancy is a candidate therapeutic approach to preventing preeclampsia-associated chronic inflammation.

The study by Ward et al[10] provides an experimental preeclampsia-like model in which NDPI leads to abnormal heart development and impaired adult cardiac function in the offspring. Further research is needed to demonstrate the relevance of the NDPI model for the study of preeclampsia, most crucially, whether it reproduces the key preeclampsia features of maternal hypertension and proteinuria. The clinical relevance of this study is the suggestion that neutrophil phenotyping in pregnant women could be used as guidance to monitor for potential CHDs in the fetus. This study also implies that maternal inflammatory cytokine levels could be used for the diagnosis of preeclampsia, as has been suggested previously.[15]

ARTICLE INFORMATION

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Disclosures

None.

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