Name: Rachel (Lane) Dahn

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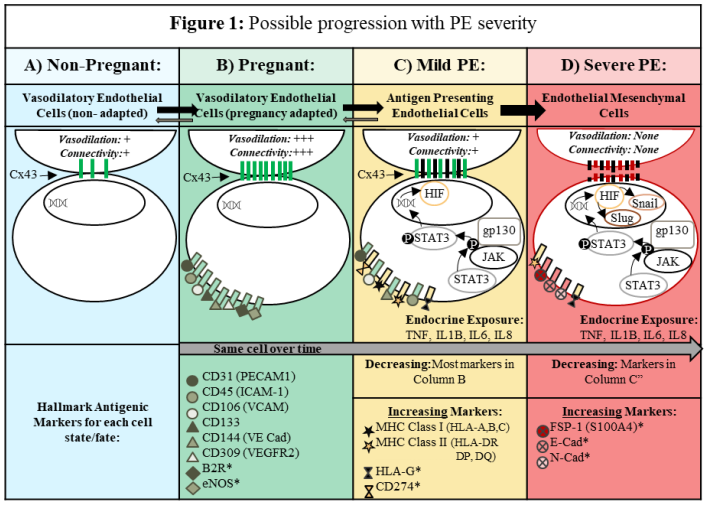
Major Professor: Ian Bird

Degree Objective: PhD, Endocrinology and Reproductive Physiology

Background: BS Biology and Animal Science, University of Findlay

Current Research Project:



Specific Aims: In healthy pregnancy, there is a high level of vascular remodeling achieved by 1) increased angiogenesis, and 2) phenotypic reprogramming including enhanced vasodilation. Enhanced vasodilation in turn is achieved via enhanced cell junctional coupling and Connexin 43 cell-cell communication. The failure, known as preeclampsia (PE), is due to what appears to be an inappropriate 'wounding' response in which excessive levels of growth factors and/or cytokines shut down cell-cell junctional coupling and Connexin closure, resulting in loss of vasodilation and a tendency to edema. Our lab has shown this is not just the action of a single factor but, variable interactions of a number of factors that *converge through a limited number of signaling pathways to drive endothelium first to an antigen presenting state and then into a Mesenchymal Transition (see Fig 1).* We propose that targeting *the cell signaling system* provides the best strategy to reverse this dysfunction. The suspected cytokines at play here are associated with a Th1 phenotype where Th1 cells dominate and TNFα, IL1B, IL6 and IL8 are elevated in vivo. We propose these cytokines have such a multilayered impact on endothelial destruction due to convergence of Src and JAK/GP130 signaling and induction of STAT3 and HIF. Preliminary phenotypic functional assays and transcriptomics data analysis over 20 hours reveals expected changes in cell-cell communication as well as changes in endocrine secretion, modification of the extracellular space (via MMPs), and alterations to immune attachment proteins (ICAM and VCAM). Further analysis suggests the onset of an antigen presenting (AP) state. The published literature also confirms the same factors acting even longer may drive an endothelial mesenchymal transition (EndoMT). To that end, we propose to determine the extent to which AP vs EndoMT cell fate changes may occur in response to TNF and the Gp130 coupled interleukins IL1B, IL6 and IL8 longer term, and the extent to which early Src and JAK signaling kinases may converge though transcription factor activation to initiate these outcomes. We begin by using our known in vitro cell systems and calibrate the cell responses to known agonists. In Aim 1 we complete the application of traditional methods to establish the activation of Src and JAK/GP130 by these cytokines alone and together, and test the rescue effects of Src and JAK inhibitors *to establish cause and effect* to verify the *mechanistic* process leading to endothelial dysfunction. In Aim 2 we will then take the effective treatment combinations in Aim 1 to gather Seq data and monitor corresponding protein biomarkers of these STAT3 and HIF mediated cell state and fate changes. In Aim 3 we will translate to the human disease, where we will apply FACS for the same biomarkers and RNA Seq to monitor cell state and fate changes in *human* endothelial cells (HUVEC) *freshly* isolated from normal vs PE pregnancy. This leads to my aims:

- **Specific Aim 1:** 1A) Establish both time and dose effects of IL6 (low through high dose) with submaximal TNF (through the use of ECIS) to pinpoint the most damaging combination. 1B) Use the dose determined in 1A to complete protein analysis with and without Src inhibitor PP2, and/or JAK inhibitor AG490, alone or together to evaluate if there is STAT phosphorylation on Tyrosine residues as well as HIF expression. 1C) Evaluate TNF effects with added cytokine, IL6, with and without inhibitors PP2 alone or AG490 alone to see rescuing ability. This includes measures of long term disruption of junctional proteins and loss of monolayer integrity via ECIS, and western blot confirmation of STAT phosphorylation/ HIF expression.
- **Specific Aim 2:** Using combined treatments determined by ECIS in Aim 1, identify the changes in transcriptome in P-UAEC using RNA-Seq, and compare to cell state related protein expression and function. Look for changes in transcriptome across a day long time course to flesh out the different cell fates; normal, antigen presenting, or endothelial mesenchymal transition.
- **Specific Aim 3:** Beginning with the findings of others and refined using data from Aim 2, develop a multidimensional FACS system to detect surface markers for antigen presenting state or for EndoMT (From Aim 2) for use in future studies. Then apply this to P-UAEC treated as in Aim 2 and then freshly isolated HUVEC from control vs PE pregnancies. Data on a) P-UAEC as treated in Aims 1C/2 and b) **Fresh** HUVEC from control vs PE pregnancy will be evaluated in order to determine specific signaling events and transcription factors driving an antigen presenting state versus the onset of an endothelial mesenchymal transition and PE symptoms. We will apply the FACS method and compare to RNA Seq data in each case.

Grants Received:

2019-21. Award to **R Dahn.** T32. Ruth L. Kirschstein National Research Service Award training grant (T32 HD041921-17). PI Ian Bird.

Ruth L. Kirschstein National Research Service Award training grant awarded to the Endocrine & Reproductive Physiology (ERP) Program by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), a pre-doctoral traineeship through the Endocrinology-Reproductive Physiology Training Grant.

2017-19. Award to **R Lane.** R25. Training and Education to Advance Minorities in Science (TEAM-Science). PI Molly Carnes.

The overall goal of the TEAM-Science Program is to develop a diverse biomedical and behavioral research workforce. In addition to increasing the numbers of URM and SWD students awarded doctorates in biomedical or behavioral disciplines, the TEAM-Science program aims to initiate enduring changes in the approach to recruitment, retention, and academic career development of graduate students at UW.

Publication in <u>Review</u>:

Ampey A, **Dahn R**, Grumer M, Bird I. (2020) Differential Control of Uterine Artery Endothelial Monolayer Integrity by TNF and VEGF is Achieved Through Multiple Mechanisms Operating Inside and Outside the Cell – Relevance to Preeclamspia. Mol Cell Endo. Submitted November 10, 2020

My role included pulling the data together, creating/unifying figures, and drafting each section of the paper.

National Presentations:

Poster Presentation: **Dahn RL**, Clemente L, Ampey AC, Austin JL, Bird IM. (2019) Src and ERK are not the only Mediators of Endothelial Dysfunction; p38MAPK also Regulates Pregnancy-Derived Uterine Artery Endothelial Monolayers. Society Reproductive Investigation - Annual Meeting, Paris, France. Abstract LB-049.

Other Presentations:

Oral Presentation: **Dahn RL**, Clemente L, Ampey AC, Austin JL, Bird IM. (2020). Understanding the complex signaling pathways and possible cell fate changes in endothelial cells as mediators in the pathogenesis of preeclampsia. Endocrinology and Reproductive Physiology Seminar and Preliminary Examination. September 10, 2020.

Poster Presentation: **Dahn RL**, Clemente L, Ampey AC, Austin JL, Bird IM. (2020) Multiple Kinases Mediate TNFα- Induced Endothelial Damage in Uterine Artery Endothelial Monolayers; Could Complexity = Opportunity? Endocrinology and Reproductive Physiology Symposium. June 4, 2020.

Oral Presentation: **Dahn RL**, Clemente L, Ampey AC, Austin JL, Bird IM. (2019) Src and ERK are not the only Mediators of Endothelial Dysfunction; p38MAPK also Regulates Pregnancy-Derived Uterine Artery Endothelial Monolayers. Madison Scholars Symposium May 8, 2019

Poster Presentation: **Dahn RL**, Clemente L, Ampey AC, Austin JL, Bird IM. (2019) Src and ERK are not the only Mediators of Endothelial Dysfunction; p38MAPK also Regulates Pregnancy-Derived Uterine Artery Endothelial Monolayers. Endocrinology Reproductive Physiology Symposium April 18, 2019.

Oral Presentation: **Dahn RL**, Clemente L, Ampey AC, Austin JL, Bird IM. (2018) Investigating Endothelial Dysfunction in Preeclampsia. Endocrinology Reproductive Physiology Seminar Dec 6, 2018.

ERP Service:

ERP ambassador for the very first Madison Scholars Science of Aging Symposium. I assisted at the event, and volunteered as one of the speakers for this new symposium representing programs blessed with T32 funding. This will hopefully turn into an annual event and serve as a symbol of gratitude and progress marker to show NIH how UW-Madison T32 programs are thriving.

ERP nomination committee for the 2020 Excellence in Graduate Academic Services and Advising Award. Helped gather, organize, and unite the individual success stories from the student body into a formal nomination letter for the candidate.

Chief Diversity and Inclusion Officer Search Committee for the student panel for the selection of the new Chief Diversity and Inclusion Officer for the SMPH program at UW-Madison. Position includes meeting perspective candidates through the interview process and making recommendations to the student and administrative groups. This interview process is still ongoing due to COVID-19.