Role of Innate Immune Macrophages in Pregnancy-Induced Hypertension

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Background & Rationale

Preeclampsia is a pregnancy specific condition characterized by:
Abnormal maternal arterial remodeling that results in placental insufficiency and placental ischemia
Onset of high blood pressure (hypertension) and often proteinuria
Increased innate immune system activation in circulation, placenta, and kidney compared to normal pregnancy (Derzy et al. 2010).

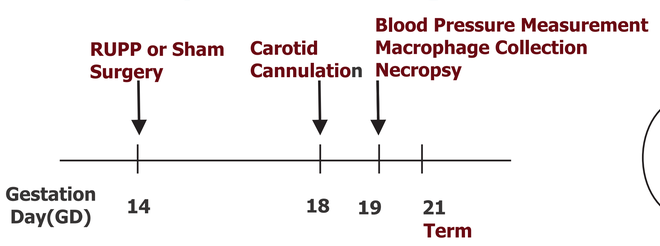
Hypertension in Non-Pregnant Animals: Hypertension is a chronic condition that affects the heart's ability to sufficiently pump blood throughout the body. Hypertension and activation of the immune system are associated with recent studies showing a specific association with **macrophages** and the development of hypertension (Rucker and Crowley 2017)

Macrophages are large, phagocytic white blood cells that have the ability to attack foreign cells and unhealthy self cells. Pro-inflammatory cytokines produced by macrophages contribute to blood pressure elevation and subsequent tissue damage (Rucker and Crowley 2017). Macrophages can be further differentiated into **M1** and **M2** macrophages.

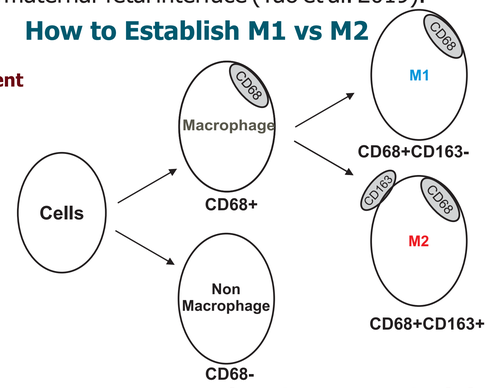
M1 versus M2 Macrophages: M1 macrophages are classically activated, whereas M2 macrophages are activated through an alternative pathway. The two types of macrophages have different phenotypes and can be distinguished by the cell markers CD163 and CD68 (Yao et al. 2019).
CD68 is a well known intracellular macrophage and monocyte marker
CD163 is a specific macrophage marker, which is distinctive to M2 macrophages

Macrophage Changes with Pregnancy: Macrophages that are present at the maternal-fetal interface at the placenta shift from M1 macrophages into M2 macrophages throughout normal pregnancy (Yao et al. 2019). In pre-eclampsia, there is evidence to suggest that **M1 macrophages increase** at the maternal-fetal interface (Yao et al. 2019).

Experimental Design

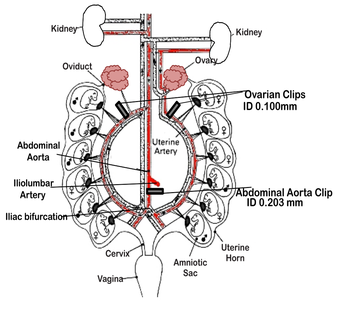


How to Establish M1 vs M2



Model of Placental Ischemia

Reduced Uterine Perfusion Pressure (RUPP) Model in the Rat:
The RUPP model is shown to increase blood pressure in pregnant rats by the addition of silver clips on the lower abdominal aorta and ovarian arteries on gestation day (GD) 14 in anesthetized Sprague Dawley rats (Charles River). This decreases blood flow to the placenta and increases the maternal blood pressure. A Sham surgery is performed as a control procedure. Blood pressure is measured from the carotid artery in a restrained, conscious rat on GD19.



Modified from Even, M et al (1992)

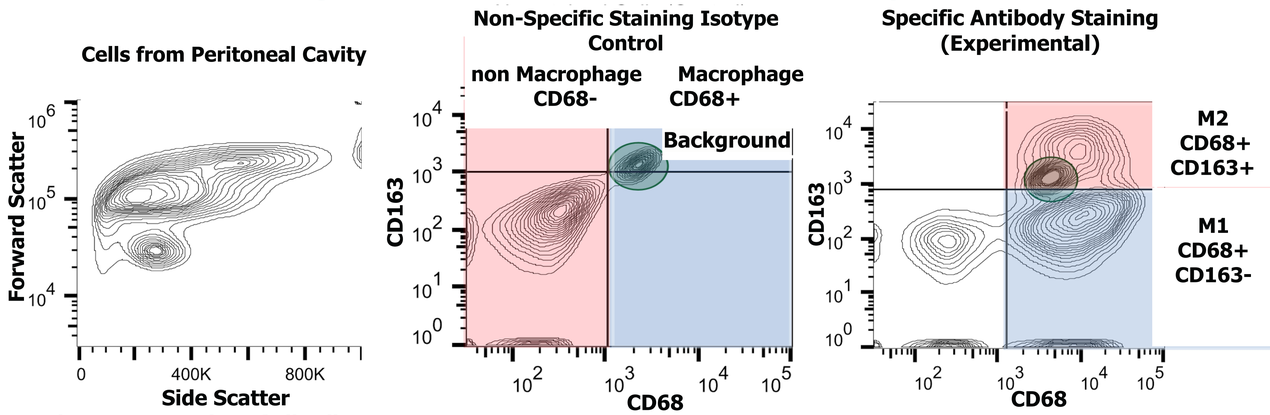
Flow Cytometry Staining
Macrophages are collected from the pregnant rat on GD19, separated by density gradient and then stained with specific antibodies. The peritoneal cells are stained with CD163 antibody or isotype control antibody marker first, then permeabilized to allow for intracellular CD68 antibody staining. Cells from the blood and placenta are only stained with CD68, since presence of CD163 is not as strong in these tissues. Number of cells stained with each marker is then determined using an Accuri flow cytometer.

References:
Even MD, Dhar MG, Vom Saal FS. Transport of steroids between fetuses via amniotic fluid in relation to the intrauterine position phenomenon in rats. Reproduction. 1992;96(2):709-16.
Derzsy Z, Prohaszka Z, Rigo J Jr, Fust G, Molvarec A. Activation of the complement system in normal pregnancy and preeclampsia. Mol Immunol. 2010;47(7–8):1500–6.
Rucker, JA, Crowley, S. The Role of Macrophages in Hypertension and Its Complications. Pflügers Archiv - European Journal of Physiology 2017;469(3-4): 419-30.
Yao Y, Xu XH, Jin L. Macrophage Polarization in Physiological and Pathological Pregnancy. Front Immunol. 2019 Apr 15;10:792.

Hypothesis

Placental ischemia results in macrophage movement from peritoneal cavity to the site of ischemia in the placenta

Quantitating Macrophages via Flow Cytometry

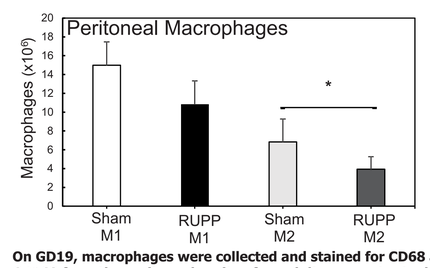


The representation of all cell events collected on the flow cytometer in terms of size (forward scatter) and complexity (side scatter). Density of cell populations indicated by lines.

Non-specific (isotype control) staining shows cells that are not stained with CD68. Isotype staining defines cell populations that are false positive (background).

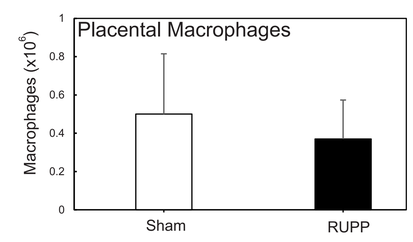
Establish the measurement of M1 vs. M2 Cells. Background signal is removed.

M2 Macrophages Decrease in the Peritoneal Cavity

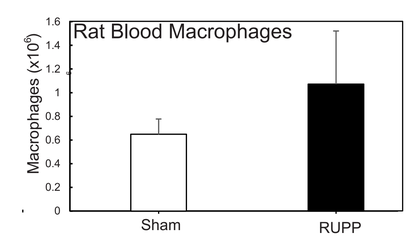


On GD19, macrophages were collected and stained for CD68 and CD163 from the peritoneal cavity of an adult pregnant rat. The presence of M2 macrophages significantly decreases. M1 macrophages show no significant change during this time (N=9/treatment group; * p<0.05)

No Change in Placental or Blood Macrophages



On GD19, macrophages were collected and stained for CD68 from two placenta of an adult pregnant rat. Macrophages show no significant change (N=4/treatment group; * p<0.05)



On GD19, macrophages from 1mL of blood from an adult pregnant rat were collected and stained for CD68. Macrophages show no significant change (N=5/treatment group; * p<0.05)

Conclusions and Future Directions

Following placental ischemia, M2 cells decrease within the peritoneal cavity.

Macrophages do not show significant changes in blood or placenta. No CD163+ signal was detected in placenta or blood suggesting no detectable M2 macrophages.

These data indicate that peritoneal macrophages do not move to the placenta following ischemia.

Future experiments will determine if macrophage depletion has a major impact on hypertension and reduced fetal growth following placental ischemia.