Identification of Natural Killer Cells in Rat Placenta Model of Preeclampsia

APPLICATION NOTE

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IN THIS PAPER YOU WILL LEARN

The preparation of Natural Killer cells from tissue

The method to measure NK activation

Introduction

Historically, the rat has been the predominant preclinical model system employed by academia and industry alike. An important area of study utilizing the rat is reproduction. A condition affecting a significant percentage of pregnancies, preeclampsia, is a complication defined by hypertension in a woman who had otherwise normal blood pressure. Preeclampsia is often related to chronic inflammation that can endanger the life of both mother and child. Utilizing a pregnant rat model to study the immune response occurring in the placenta can aid our understanding of preeclampsia, with the ultimate goal of developing a therapy to correct the pathophysiology of this serious condition. Here, we characterize the presence of Natural Killer cells in rat placenta.

Materials & Methods

Pregnant mice on day 19 of gestation were euthanized and placentas were harvested. Single cell suspensions were obtained from the placentas. Cells were incubated for 30 minutes at 4°C with or without an antibody against ANK61, a marker of Natural Killer cells. Then both conditions were subsequently washed 3 times by centrifugation at 400 g for 5 minutes and incubated with secondary FITC for 30 minutes at 4°C in the dark. Lastly, these cells were washed 3 times and resuspended in RPMI containing 50% formalin. Cells were analyzed on a CytoFLEX* flow cytometer. The percentage of positive staining cells above the negative control was then analyzed.

Reagents

ANK61 mouse-anti rat monoclonal purified antibody / Abcam ab36392.
Goat anti-mouse FITC / Abcam ab6785.

Data Analysis

FSC-A x SSC-A live gate
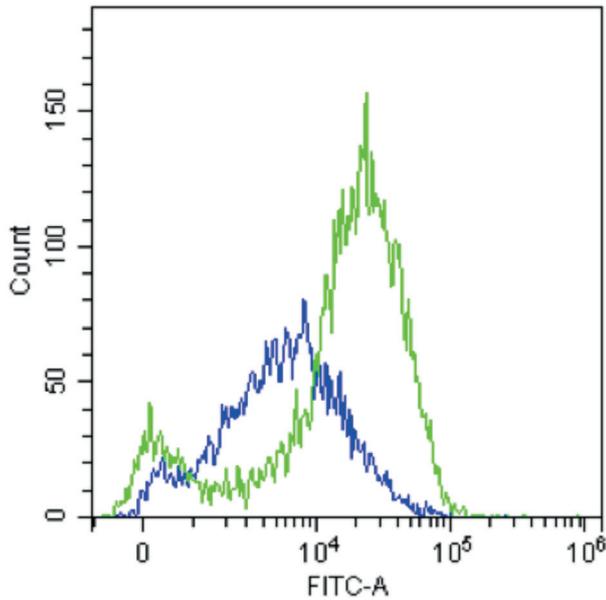
Figure 1. Overlay histogram of secondary goat anti-mouse FITC (blue) and ANK61 mouse-anti rat monoclonal purified antibody followed by goat anti-mouse FITC (green).

Results

Here, we demonstrate an increase in activated NK cells from rat placenta using the CytoFLEX, allowing us to further characterize the immune response occurring in the placenta with the ultimate goal to further understand of the pathophysiology preeclampsia.

Reference

Amaral LM, Cunningham Jr MW, Cornelius DC, LaMarca B. Preeclampsia: long term consequences for vascular health. Vasc Health Risk Manag. 2015 in press.

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