

Effects of amniotic fluid inoculated in the pregnant uterus and its proteomic analysis

Vivian B Ribeiro, Aline F P Mello, Daniel M Souza, Aureo T Yamada

Abstract

Pregnancy specific uterine natural killer (uNK) cells subset accumulate in the uterus and play critical roles in the vascular remodeling during hemochorial placentation. However, their cytolytic activity related to innate immune response inducing pregnancy loss has not experimentally established. The present work hypothesised the releasing of amniotic fluid by breaking of chorioamniotic barrier could activate uNK cells cytolytic response.

Key words: *uterine NK cell, innate immune response, immunology of reproduction*

Introduction

Migration and differentiation of pregnancy specific natural killer cells in the uterus (uNK cells) is critical for endometrial angiogenesis, spiral artery modification, modulation of immune response and hemochorial type placentation both in human and rodents. However, the releasing of cytolytic contents from the granules of fully differentiated uNK cells to provoke the miscarriage and recurrent pregnancy loss of unknown etiology, and the triggering mechanism of uNK cell cytotoxic activity is speculative. Previously, we reported the intrauterine surgical euthanasia of embryo induced degranulation of uNK cells(1). The present work aimed to evaluate the same effect of amniotic fluid leakage in the mouse uNK cell and further identification of candidate mediators of uNK cell cytotoxic activation by proteomic analysis of amniotic fluid.

Results and Discussion

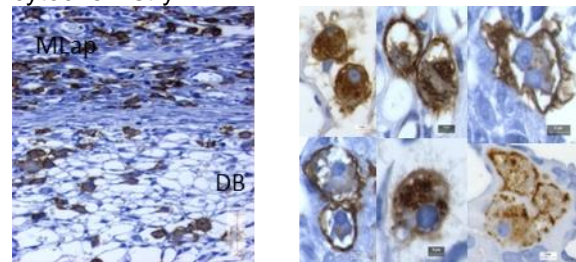
Methods: Amniotic fluid (AF) was collected from each embryo developing site at gestational day (gd) 12 and 17 and, pooled for each animal to inoculate 2µL at least in two embryo inter-implantation sites through the antimesometrial side of uterine horn at gd 9, 12 and 17. After 60 min, the uterine horns were dissected and processed for conventional paraffin embedding and *Dolichos biflorus* (DBA) lectin cytochemistry were performed on histological sections. The remainder amniotic fluid were frozen and stored in N2 for proteomic analysis.

Results and Discussion:

DBA lectin cytochemistry is the gold standard to identify the mouse uNK cell subtypes based on cell surface and cytoplasmic granules labeling (2). The control group without AF inoculation identified the uNK cells subtypes I, II, III and IV in the mesometrial lymphoid aggregates associated to pregnancy (MLAp) and decidua basalis (DB) at gd 9, 12 and 17 as were reported (2). The AF inoculation induced change on DBA lectin labeling pattern of uNK cells localized in the MLAP and DB

of all samples analysed, being the most frequent pattern those cells lost the granule labeling and disrupted labeling on cell surface of mature (subtypes III and IV) uNK cells. Those immature subtypes I and II uNK cells showed stronger labeling on cell surface and cytoplasm. This pattern suggest releasing of granule contents from mature subtypes and differentiation of immature subtypes under AF stimulus. Similar changes of uNK cells labeling pattern also seen in the MLAP and DB of embryo developing not inoculated with AF suggests systemic effects. Therefore uNK cells are sensitive to changes in the homeostasis of uterine environment simulated by leakage of AF and triggers its innate immune type response. This study will further investigate the AF contents by proteomic analysis to identify the mediators capable to activate such a uNK cell response.

Image 1. Photomicrographies of mouse uNK cell distribution in the MLAP and DB details of morphological changes induced by AF. DBA lectin cytochemistry



Conclusions

Mouse uNK cells are sensitive to amniotic fluid and activate their innate immune type response by releasing cytolytic granules contents.

Acknowledgement

Ribeiro VB was recipient of PIBIC/UNICAMP 2015 scholarship.

1-PDA Lima, BA Croy, KY Degaki, C Tayade, Heterogeneity in composition of mouse uterine natural killer cell granules. *AT Yamada. Journal of leukocyte biology* 92 (1), 195-204, 2013
2- BA Croy, J Zhang, C Tayade, F Colucci, H Yadi AT Yamada. Analysis of uterine natural killer cell. *IN: Natural Killer Cell Protocols*, 2010. DOI 10.1007/978-1-60761-362-6_31