

A PROTEOMIC APPROACH TO IDENTIFYING PEPTIDES WITH POTENTIAL TO PROMOTE TRAFFICKING OF NATURAL KILLER (NK) CELLS TO THE UTERUS

Crystal Peralta¹, Marianne van den Heuvel², Kota Hatta³, B. Anne Croy⁴, and Victor Han².

Depts. of ¹Anatomy and Cell Biology, ²Pediatrics, and ³Microbiology and Immunology, University of Western Ontario, London ON N6A 5B8; ⁴Anatomy and Cell Biology, Queen's University, Kingston, ON K7L 3N6.

INTRODUCTION: A dramatic shift in immune cell populations occurs in human uterus immediately after ovulation. This coincides with the differentiation of endometrial stromal cells into decidual cells, a process essential for blastocyst implantation. The dominant lymphocytes in early decidua are CD56^{bright} NK cells that lack CD16 and have limited lytic activity. Decidual (d)NK cells arise by precursor homing and proliferation in the uterus. They secrete numerous cytokines and growth factors (IFN- γ , LIF, VEGF), thereby contributing to implantation, endometrial neovascularization and menstruation. Human blood NK cells from fertile, but not infertile women, show a transient, peri-ovulatory gain in L-selectin based adhesive function under shear forces to mouse decidual endothelium *in vitro*. However, this change in trafficking potential is not associated with altered expression of L-selectin, $\alpha 4$ integrin, LFA-1, CXCR3, or CXCR4 by CD56^{bright} NK cells and remains undefined.

OBJECTIVE To identify the molecules responsible for altered trafficking potential of blood NK cells in the post ovulatory phase of the menstrual cycle.

METHODS: Paired blood samples were collected from 18 female volunteers of known fertility at cycle day (cd) 5 (lower adhesion) and day of ovulation (OD; higher adhesion). Three CD56⁺ subsets were prepared using AutoMacs separation: CD56^{bright} (CD56⁺CD16⁻), CD56^{dim} (CD56⁺CD16⁺) and NKT (CD56⁺CD3⁺) cells. Using buffered detergents, membrane and organelle protein fractions were collected from each sample. Membrane samples from individual subsets at the same time point were pooled from 10 subjects to obtain sufficient protein for 2D-PAGE analysis. This technique was repeated using pooled samples from 8 different subjects. Analysis software, Phoretix 2D Expression, was used to compare protein expression from cd5 samples with OD samples. Differentially expressed proteins were further analyzed by mass spectrometry.

RESULTS AND CONCLUSIONS: Comparing the expression of proteins at the time of LH surge versus cd5 in each of the three subsets show a number of proteins with a greater than 2 fold change in normalized volume expression: CD56^{bright} (27 upregulated, 34 downregulated), CD56^{dim} (118 upregulated, 11 down regulated), and NKT (25 upregulated, 71 down regulated). Identification of these protein spots continues and will be reported. Changes in CD56⁺ cell protein expression at ovulation are expected to initiate NK cell trafficking to the uterus at the LH surge. This information may assist in subset classification of infertile women.

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