Preterm human milk exosomes have anti-inflammatory properties mediated by miRNA-146a that is decreased in donor milk

F. Asaad; X. Lin; C. Manzano De Meja; M. Hanna; N. Hanna
Department of Pediatrics, NYU Winthrop University Hospital, NY, USA

**Background**

- Breast milk (BM) contains protective components against inflammatory injury
- Exosome vesicles found in BM are released in response to various stimuli
- Exosomes contain cytokines as well as microRNAs (miRNA), involved in various biological processes through regulation of posttranscriptional gene expression
- miR-146a was shown to be involved in immune tolerance by down-regulation of TNF-α

**Objectives**

- To examine whether preterm and term BM contains exosomes that contribute to anti-inflammatory regulation in preterm infants, as compared to donor milk (DM)

**Methods**

- Term, preterm (PT) BM samples (<32 weeks gestation) were collected in week 1 and 4 after birth, term donor (DM) samples and exosomes were isolated using differential ultracentrifugation method
- Level of exosomes in BM was determined by total protein recovery and exosome specific ELISA, and cytokine levels in exosomes were determined by ELISA
- Exosomes were co-cultured with THP-1 derived macrophages exposed to LPS. The supernatants were analyzed for TNF-α production
- We analyzed expression of selected miRNA in BM and DM exosomes using RT-PCR
- THP-1 derived macrophages were transfected with miR-146a mimic, the transfected cells were stimulated with LPS, and TNF-α expression and secretion were determined by qPCR and ELISA

**Results**

- Figure 1. EV number in BM (term and preterm) and donor
- Figure 2. Exosomes from DM induced TNF-α in THP-1 cells more than preterm samples
- Figure 3. miRNAs content of EVs (miR-146a)
- Figure 4. transfection of miR-146a mimic inhibits TNF-α expression

**Conclusions**

- Exosomes are produced in Term, PT BM and DM, which was confirmed via electron microscopy, appearing as cup shaped with a diameter around 100nm.
- Exosomes isolated from PT BM inhibited LPS induced TNF-α production in macrophages. However exosomes isolated from DM exaggerated the TNF-α production
- DM exosomes had decreased miRNAs content, whereas PT and term exosomes contained several miRNAs including the anti-inflammatory miRNA-146a
- Exosomal miRNA-146a was significantly higher in term and PT milk compared to DM
- miRNA-146a transfection inhibited LPS induced TNF-α gene expression and protein secretion in macrophages

**Future Studies**

- Study whether BM derived exosomes can bind to gastrointestinal cells
- Study whether BM derived exosomes can modify gastrointestinal cell differentiation and their responses to infectious stimuli

**Figure 1. EV number in BM (term and preterm) and donor**

<table>
<thead>
<tr>
<th>EVs from BM</th>
<th>Term wk1</th>
<th>PT wk1</th>
<th>PT wk4</th>
<th>Donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (mg/mL)</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

**Figure 2. Exosomes from DM induced TNF-α in THP-1 cells more than preterm samples**

<table>
<thead>
<tr>
<th>BM</th>
<th>Term</th>
<th>PT wk1</th>
<th>PT wk4</th>
<th>Donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGFα (pg/mL)</td>
<td>2000</td>
<td>2000</td>
<td>1500</td>
<td>1000</td>
</tr>
</tbody>
</table>

**Figure 3. miRNAs content of EVs (miR-146a)**

<table>
<thead>
<tr>
<th>miR-146a</th>
<th>miR-146a, DCt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Term</td>
<td>10.0</td>
</tr>
<tr>
<td>Preterm</td>
<td>12.0</td>
</tr>
<tr>
<td>Donor</td>
<td>15.0</td>
</tr>
</tbody>
</table>

**Figure 4. transfection of miR-146a mimic inhibits TNF-α expression**

<table>
<thead>
<tr>
<th>Neg</th>
<th>miR-146a mimic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fold Change</td>
<td>1.0</td>
</tr>
<tr>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

**Department of Pediatrics, NYU Winthrop University Hospital, NY, USA**

**Figure 3. miRNAs content of EVs (miR-146a)**

- Term, preterm (PT) BM samples (<32 weeks gestation) were collected in week 1 and 4 after birth, term donor (DM) samples and exosomes were isolated using differential ultracentrifugation method
- Level of exosomes in BM was determined by total protein recovery and exosome specific ELISA, and cytokine levels in exosomes were determined by ELISA
- Exosomes were co-cultured with THP-1 derived macrophages exposed to LPS. The supernatants were analyzed for TNF-α production
- We analyzed expression of selected miRNA in BM and DM exosomes using RT-PCR
- THP-1 derived macrophages were transfected with miR-146a mimic, the transfected cells were stimulated with LPS, and TNF-α expression and secretion were determined by qPCR and ELISA

**Results**

- Figure 1. EV number in BM (term and preterm) and donor
- Figure 2. Exosomes from DM induced TNF-α in THP-1 cells more than preterm samples
- Figure 3. miRNAs content of EVs (miR-146a)
- Figure 4. transfection of miR-146a mimic inhibits TNF-α expression

**Conclusions**

- Exosomes are produced in Term, PT BM and DM, which was confirmed via electron microscopy, appearing as cup shaped with a diameter around 100nm.
- Exosomes isolated from PT BM inhibited LPS induced TNF-α production in macrophages. However exosomes isolated from DM exaggerated the TNF-α production
- DM exosomes had decreased miRNAs content, whereas PT and term exosomes contained several miRNAs including the anti-inflammatory miRNA-146a
- Exosomal miRNA-146a was significantly higher in term and PT milk compared to DM
- miRNA-146a transfection inhibited LPS induced TNF-α gene expression and protein secretion in macrophages

**Future Studies**

- Study whether BM derived exosomes can bind to gastrointestinal cells
- Study whether BM derived exosomes can modify gastrointestinal cell differentiation and their responses to infectious stimuli