HUMAN NEONATES DISPLAY ALTERED EX VIVO MONOKINE PRODUCTION RELATED TO HEALTHY ADULTS.

Paulo RZ Antas, PhD
Lab. de Imunologia Clínica / IOC / FIOCRUZ
Contributors

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• Eliana A. Santiago¹
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Supported by: FAPERJ fellowships; CNPq-PQ-2 fellowship and Fiocruz.
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Disclosure

None of the authors have a commercial association that poses a conflict of interest in relation to this program/presentation.
Background

In vitro T-cell profile induced by BCG Moreau in healthy Brazilian volunteers

C Ponte, L. Peres, S. Marinho, J. Lima, M. Siqueira, T. Pedro, P. De Luca, C. Cascalho, L.R. Castello-Branco, and P. R. Z. Antunes

Laboratório de Imunologia Clínica, Instituto Oswald Cruz, Fiocruz, Rio de Janeiro, Brazil; Laboratório de Imunoparasitologia, Instituto Oswald Cruz, Fiocruz, Rio de Janeiro, Brazil; Laboratório de Inovações em Terapias, Enxertos e Bioprodutos, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil

Figure 2. Cytokine productions induced by BCG Moreau infection at 48 h in PBMCs from healthy donor (HD) and CBMCs from umbilical vein (UV) individuals. Shown are levels of (A) IL-2, (B) IFN-γ, (C) TNF-α, and (D) IL-10 in (pg/mL). Bars depict the mean levels in each condition. *P < 0.05; **P < 0.01.
Background

In vitro T-cell profile induced by BCG Moreau in healthy Brazilian volunteers

Figure 4. (A) Transforming growth factor (TGF)-β1 levels in pg/mL measured by ELISA in the supernatants of PBMCs from healthy donor (HD) and CBMCs from umbilical vein (UV) individuals stimulated with BCG Moreau for 48h. Horizontal bars represent mean values in each condition. (B) Tr1 cells (CD4+IL-10+FOXP3+; open bars) and monocytes+IL-10+ (black bars) induced by BCG Moreau at 48 h in P8MC from healthy donor. Bars depict the mean levels (+ SEM) in each condition. **P < 0.01.
• There is a high global burden of Inf. Dis. in the very young.
• Immunity is not static; it changes with age, with many distinctive features in early life.
• Newborns and young infants have distinct immune ontogeny and responses to microbes.

Dowling & Levy. Trend in Immunology 2014 35(7), 299-310

• Newborns exhibit increased susceptibility to infectious agents;
• Generalized hypofunction of inflammatory and immune mechanisms, related to the natural dampening of the Th-1 associated immune response, increasing the risk of infection in this exposed population.
• The neonatal immune system is constantly maturing, but, there are virtually no comparative studies concerning ex vivo broaden analysis addressing the role of monokines in the newborn vulnerable population.
Background

**Plasma**
- Immunosuppressive adenosine
- ADA

**NK cell immaturity**
- TGF-β-mediated suppression

**Erythroid cells**
- Immunosuppressive CD71+ cells

**Layered immune system:**
- T cell subset
  - Maternal, fetal liver
  - Liver/bone marrow
  - Bone marrow
  - Immunosuppressive epigenetic state
  - Breg suppression of DCs

**T cells**
- Tregs
  - Ag-specific tolerance
  - Inhibitory Tfh cells

**B cells**

*Dowling & Levy. Trend in Immunology 2014 35(7), 299-310*
### Background

<table>
<thead>
<tr>
<th>Plasma</th>
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<tbody>
<tr>
<td>Fetal</td>
<td>TGF-β-mediated suppression</td>
</tr>
<tr>
<td>Preterm</td>
<td>TGF-β-induced NK cell immaturity</td>
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<tr>
<td>Term</td>
<td>Immunosuppressive CD71+ cells</td>
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<tr>
<td>1-2 yr infant</td>
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</table>

- **NK cell immaturity**
- **Erythroid cells**

- **Layered immune system:**
  - T cell subset
  - Origin
  - Adaptive bias

- **T cells**
  - Tregs
  - Maternal, fetal liver
  - Ag-specific tolerance

- **B cells**
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*Dowling & Levy. Trend in Immunology 2014 35(7), 299-310*
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### Background

Human Immunology 60, 331–336 (1999)

American Society for Histocompatibility and Immunogenetics, 1999

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Analysis of the Cytokine Production by Cord and Adult Blood

S. B. A. Cohen, I. Perez-Cruz, P. and J. A. Madrigal

#### TABLE 1 Summary of comparisons between adult and cord blood cytokine production.

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<tr>
<th>Cells</th>
<th>Stimulation</th>
<th>Analysis</th>
<th>Adult Blood</th>
<th>Cord Blood</th>
<th>Reference</th>
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<tr>
<td>CD45RA+ T cells</td>
<td>αCD2+αCD28+ PMA</td>
<td>ELISA</td>
<td>++</td>
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<tr>
<td>Freezing/alleantigen</td>
<td>ELISA</td>
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<td>++</td>
<td>++</td>
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<td>PMA plus lomomycin</td>
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<td>++</td>
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<td>MIP-1α</td>
<td>++</td>
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</table>

*Abbreviations:* ELISA = enzyme-linked immunosorbent assay, PMA = phorbol 12-myristate 13-acetate, HTLp = human T-cell line, IFNγ = interferon-γ, TGF-β1 = transforming growth factor-β1, GM-CSF = granulocyte-macrophage colony-stimulating factor, IL = interleukin, TNFα = tumor necrosis factor-α, LPS = lipopolysaccharide, MIP-1α = macrophage inflammatory protein-1α.
Background

Letter to the Editor

Comments on the elevated IL-27 expression in neonates: Relevance between detecting nucleotide sequence or protein synthesized

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Immunology Letters 164 (2015) 53–54

Fig. 1. The ex vivo human IL-27 levels (pg/ml) was determined in thawed healthy donor adult plasma (HD: n=52) and neonate umbilical cord blood samples (UCB: n=51) using a commercially available enzyme linked immunosorbent assay (ELISA) kit (DuoSet, R&D Systems, USA). The immunoassay was carried out according to the manufacturer instructions, the detection limit was 86 pg/ml, and horizontal bars represent mean values. *p < 0.001.
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Rational

- To reveal critical aspects of *ex vivo* monokine and lymphokine profiles related to both innate and adaptive immunity in a community based open-label cross-sectional population study of a Brazilian sample.

The study was undertaken to compare newborn (UV) and adult (HD) plasma samples using multiplex array and ELISA approaches, and we set out to investigate whether the quantitative detection of circulating biomarkers differs between these groups.
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- Exclusion criteria: HIV-seronegative status, a negative history of malignant, degenerative, or transmitted diseases, diabetes mellitus, and use of corticosteroids or other immunosuppressive agents at the time of the study entry.
- Subjects' identities were omitted.
- The study was approved by the Institutional Review Board of the State University Hospital (#060/2009 & #089/2011).
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Methods

- Fresh venous (HD) or cord (UV) blood.
- 2 vials of plasma kept at -70 °C.
- Extensive evaluations of pro- and anti-inflammatory pathway cytokines (biomarkers) by:
  - Protein multiarray system (Bio-Rad, Hercules, CA, USA) to quantify human IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, GM-CSF, TNFα and IFNγ.
  - ELISA (DuoSet R&D, Minneapolis, MN, USA) to quantify human IL-1α, IL-18, IL-23, IL-27, IL-33 and TGF-β1 in parallel.
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HD=28
UV=28
Table 1: Characteristics of the neonate population.

<table>
<thead>
<tr>
<th>Neonatal growth parameters</th>
<th>UV</th>
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<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>39.2 ± 0.07a</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>51.1 ± 0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mode of delivery</th>
<th>UV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induced vaginal</td>
<td>ND</td>
</tr>
<tr>
<td>Vaginal</td>
<td>2 (13%)b</td>
</tr>
<tr>
<td>Elective cesarean</td>
<td>12 (80%)</td>
</tr>
<tr>
<td>Emergency cesarean</td>
<td>1 (7%)c</td>
</tr>
<tr>
<td>NA</td>
<td>13 (46%)d</td>
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</tbody>
</table>

*aMean ± SEM; bDuration: 5.3h; cDuration: 4h; dIRB restrictions.*
Table 2: *Ex vivo* human cytokine levels (pg/ml) determined in thawed healthy donor adult plasma (HD=28) and umbilical cord blood samples (UV=28) using commercially available protein multiarray system and enzyme linked immunosorbent assay (ELISA).

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>UV</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1a</td>
<td>0.07 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>IL-2</td>
<td>7.7 ± 3.2</td>
<td>4.6 ± 2.1</td>
</tr>
<tr>
<td>IL-4</td>
<td>29.4 ± 9.0</td>
<td>17.1 ± 7.3</td>
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<td>IL-5</td>
<td>27.3 ± 4.9</td>
<td>24.7 ± 3.6</td>
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<td>IL-10</td>
<td>77.2 ± 23.9</td>
<td>27.6 ± 9.5</td>
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<td>IL-12</td>
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<td>IL-33</td>
<td>0.02 ± 0.0</td>
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<tr>
<td>IFNγ</td>
<td>69.8 ± 15.5</td>
<td>51.8 ± 12.0</td>
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<td>TNFα</td>
<td>46.2 ± 12.8</td>
<td>32.4 ± 9.1</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>22.3 ± 7.7</td>
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<sup>a</sup>Mean ± SEM;

<sup>b</sup>p < 0.0001, when compared to HD group and based on statistical significance using the Mann-Whitney U test.
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<td>27.3 ± 4.9</td>
<td>24.7 ± 3.6</td>
</tr>
<tr>
<td>IL-10</td>
<td>77.2 ± 23.9</td>
<td>27.6 ± 9.5</td>
</tr>
<tr>
<td>IL-12</td>
<td>11.7 ± 4.2</td>
<td>7.9 ± 1.5</td>
</tr>
<tr>
<td>IL-13</td>
<td>17.9 ± 2.2</td>
<td>14.3 ± 0.9</td>
</tr>
<tr>
<td>IL-33</td>
<td>0.02 ± 0.0</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>IFNg</td>
<td>69.8 ± 15.5</td>
<td>51.8 ± 12.0</td>
</tr>
<tr>
<td>TNFa</td>
<td>46.2 ± 12.8</td>
<td>32.4 ± 9.1</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>22.3 ± 7.7</td>
<td>10.2 ± 4.0</td>
</tr>
<tr>
<td>IL-27</td>
<td>1.6 ± 0.5b</td>
<td>12.3 ± 3.4</td>
</tr>
</tbody>
</table>

*aMean ± SEM;
b*p < 0.0001, when compared to HD group and based on statistical significance using the Mann-Whitney U test.
The ex vivo human IL-18 & IL-23 levels (ng/ml) were determined in thawed healthy donor adult plasma (HD; n=28) and umbilical cord blood samples (UV; n=28) using commercially available enzyme linked immunosorbent assay (ELISA) kits.

The horizontal bars represent mean values. **p < 0.01, based on statistical significance using the Mann-Whitney U test.
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Correlation analysis intra-group (UV; n=28) of IL-18 and IL-23 plasma levels (ng/ml).

Spearman’s rank coefficient test ($\rho$).

\[ \rho = 0.52; \text{p-level} = 0.02 \]
Correlation analysis intra-group (UV; n=28) of IL-18 and IL-23 plasma levels (ng/ml).

Spearman’s rank coefficient test ($\rho$).
The ex vivo human TGF-β1 levels (ng/ml) were determined in thawed healthy donor adult plasma (HD; n=28) and umbilical cord blood samples (UV; n=28) using a commercially available enzyme linked immunosorbent assay (ELISA) kit.

The horizontal bars represent mean values. **p < 0.01, based on statistical significance using the Mann-Whitney U test.
The ex vivo human TGF-β1 levels (ng/ml) were determined in thawed healthy donor adult plasma (HD; n=28) and umbilical cord blood samples (UV; n=28) using an commercially available enzyme linked immunosorbent assay (ELISA) kit. The horizontal bars represent mean values. **p < 0.01, based on statistical significance using the Mann-Whitney U test.
**Cytokine Network**

- **Maturação de Células B**
  - IL-6
  - IL-1

- **Monócito**
  - IL-12
  - IFN-α
  - IFN-β

- **Macrôfago**
  - TNF-α
  - IL-6
  - IL-10

- **T**
  - IFN-γ
  - IL-12
  - IL-18

- **NK**
  - IFN-γ
  - IL-12

- **Produção do granuloma**
  - Defesa antibacteriana
  - Atração da resposta Th1

- **Inibição da síntese de citocinas**
  - B7.2
  - MHCII
  - B7.1

- **MIF**

- **Produção de IFN-γ**
  - Ativação da resposta Th1

- **Promove atividade de IFN-γ e fenótipo Th1**
Considerations

• Several factors may be implicated in those neonatal alterations, such as inherent immaturity or regulatory T cell-mediated inhibition.

• The apparent superior performance of the ELISA compared to the multiplex approach was an anticipated bias, due to our selective choice to quantify monokines based on own previous data.

• Previously, UV showed high IL-10 levels and/or decreased expression of the beta-2-microglobulin.
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Conclusion

- Term human newborns unveil a differential monokine production patterns when compared to healthy adults, and those variations seem to be corrected during the immune system development.
Perspective

- Additional characterization of a broader cytokine panel might reveal other future candidates linked to that common underlying mechanism in order to better understand the functional capability of the neonatal immune system.
OBRIGADO

(Thank You!)

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