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Instituto Oswaldo Cruz

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

Paulo RZ Antas, PhD

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Contributors

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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

SHORT REPORT

Human Vaccines & Immunotherapeutics 11:2, 450–457; February 2015; © 2015 Taylor & Francis Group, LLC

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

C Ponte[1,†], L Peres[1,†], S Marinho[1], J Lima[1], M Siqueira[1], T Pedro[1], P De Luca[2], C Cascabulho[3], L R Castello-Branco[1], and P R Z Antas[1,*]

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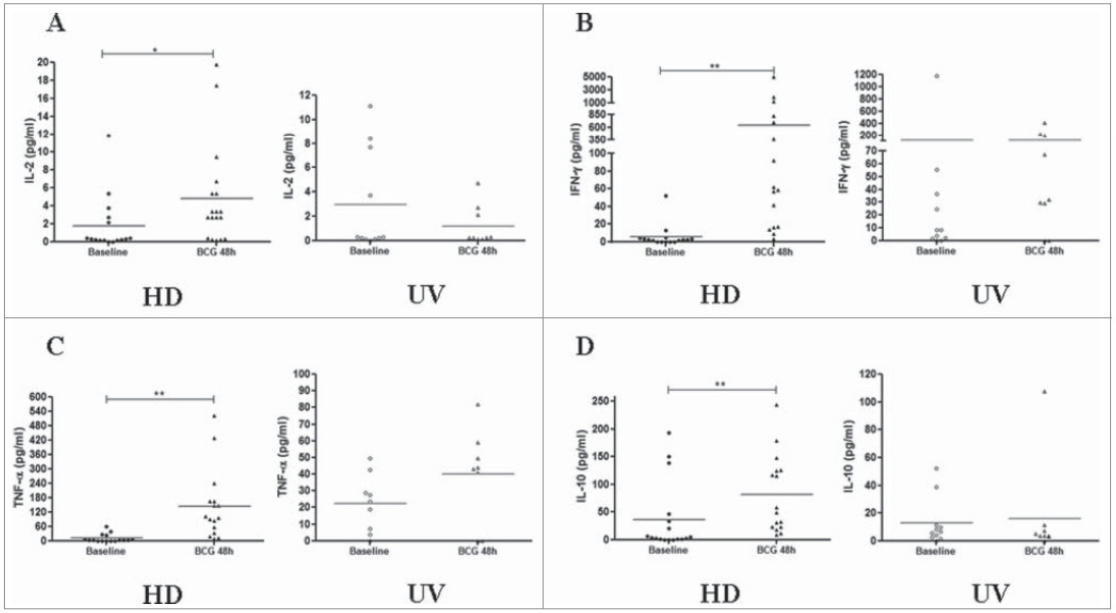

Figure 2. Cytokine productions induced by BCG Moreau infection at 48 h in PBMCs from healthy donor (HD) and CBMCs from umbilical vein (UV) individuals. Shown are levels of (**A**) IL-2, (**B**) IFN-γ, (**C**) TNF-α and (**D**) IL-10 in (pg/mL). Bars depict the mean levels in each condition. $^{*}P <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

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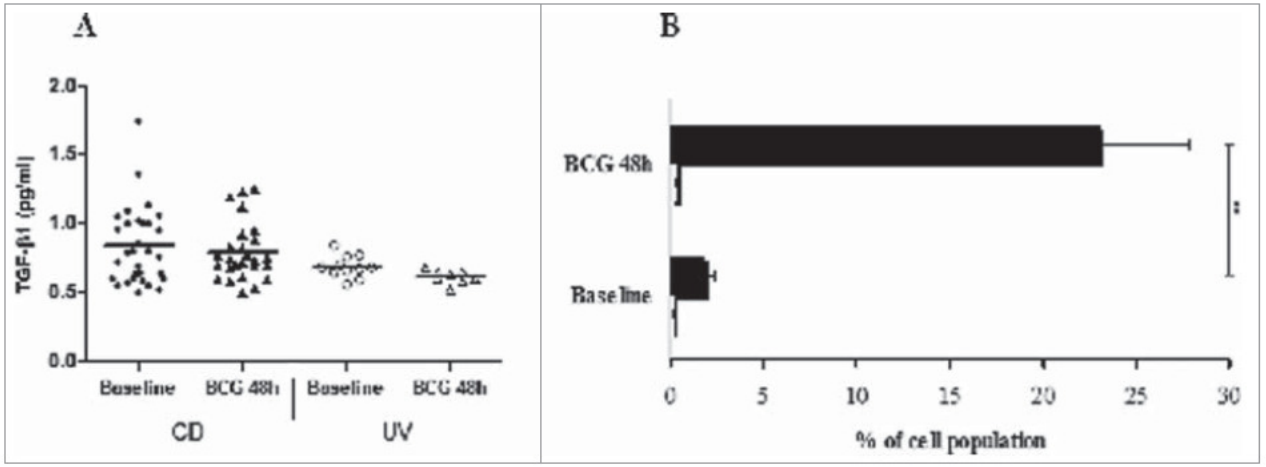

Figure 4. (**A**) Transforming growth factor (TGF)-β1 levels in pg/mL measured by ELISA in the supernatants of PBMCs from healthy donor (HD) and CBMCs from umbilical vein (UV) individuals stimulated with BCG Moreau for 48h. Horizontal bars represent mean values in each condition. (**B**) Tr1 cells (CD4+IL-10+FOXP3+; open bars) and monocytes+IL-10+ (black bars) induced by BCG Moreau at 48 h in PBMC from healthy donor. Bars depict the mean levels (+ SEM) in each condition. $^{**}P < 0.01$.

Background

- 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.

Kraft et al. Immunology 2013 139, 484-93

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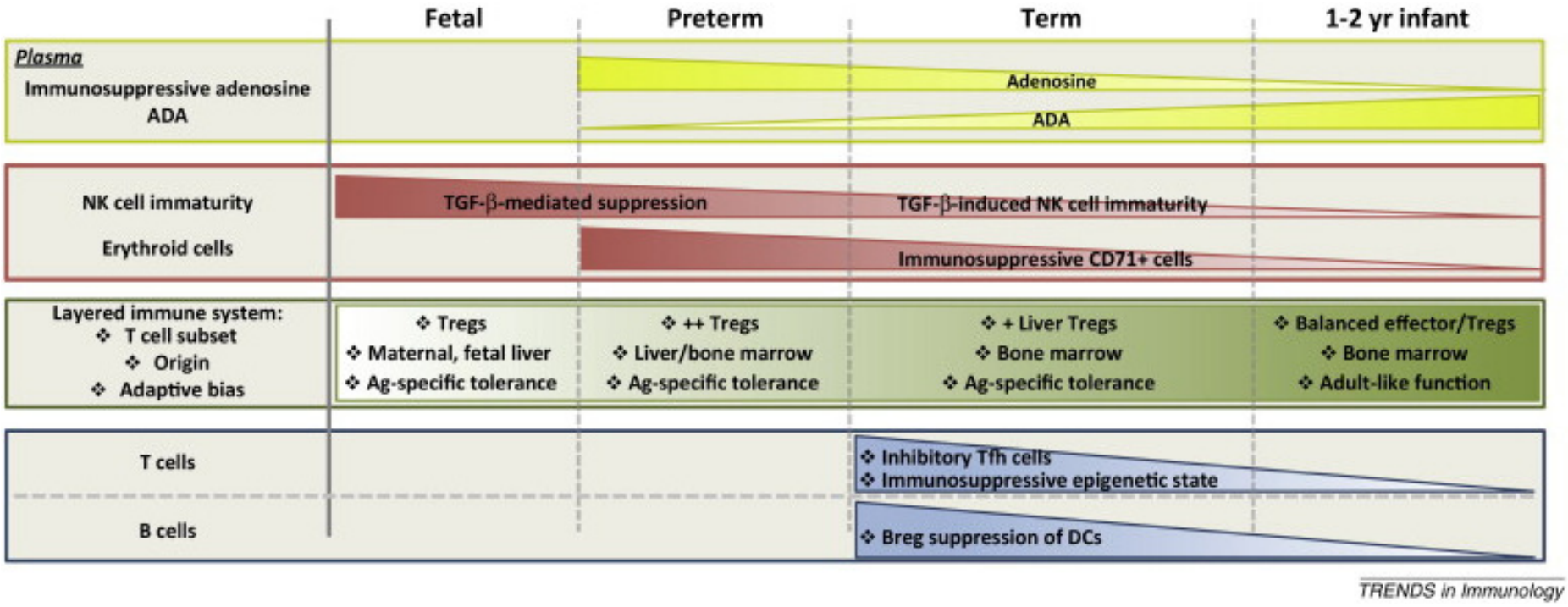

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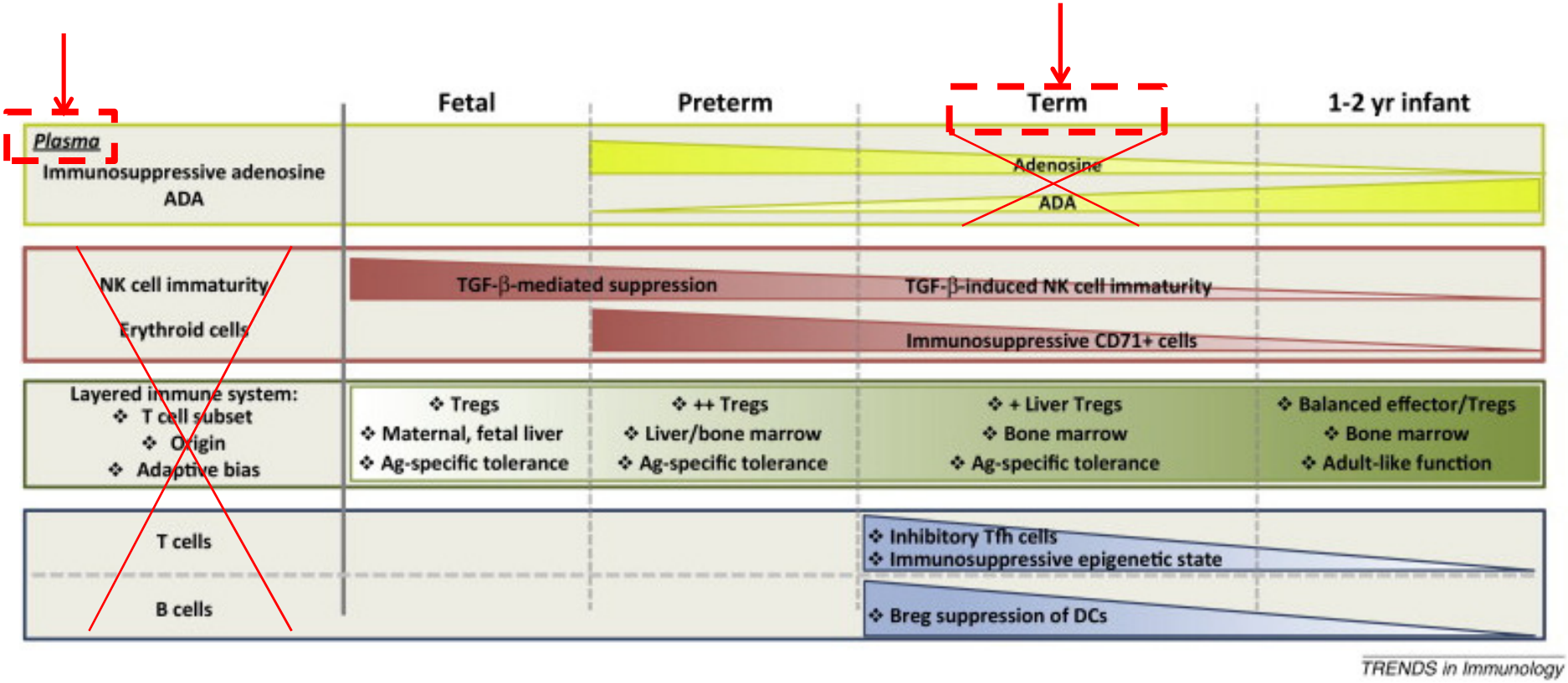

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REVIEW ARTICLE

Analysis of the Cytokine Production by Cord and Adult Blood

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

Background

Human Immunology 60, 331–336 (1999)

Published by Elsevier Science Inc.

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

Cells	Stimulation	Analysis	Cytokine	Adult Blood	Cord Blood	Reference
CD45RA+ T cells	αCD2+αCD28±[1]PMA	[2]ELISA	IL-2	++	−	[11]
	Alloantigen	HTLp	IL-2	++	++	[*]Dr. A. M. Dickinson, Personal communication
	Alloantigen	ELISA	IFNγ	+++	++	[*]Dr. A. M. Dickinson, Personal communication
	[3]PHA	ELISA/[4]mRNA	TGF-β1	++	+	[28]
	PMA/PHA	mRNA	GM-CSF	+++	+	[29]
	Freezing/alloantigen	ELISA	IL-2	+++	++	[26]
	PMA plus Ionomycin	[5]Intracellular cytokine staining	IFNγ	+++	+	[30], Drs. [*]Bottazzin & [§]Velz: Personal communication
			IL-2	+++	+	
			IL-4	+++	+	
			TNFα	+++	+	
NK cells	No stimulation	ELISA	IFNγ	+	−	[25], [31]
			IL-6	+	−	
			TNFα	+	−	
	IL-2 expansion	ELISA	IFNγ	ND	reduced	[24]
			IL-6	ND	constant	
			TNFα	ND	constant	
			IL-2R	ND	constant	
	IL-12	ELISA/mRNA	IFNγ	++	++	[25]
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	PMA/PHA	mRNA	IL-8	++	+	[28]
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Letter to the Editor

Immunology Letters 164 (2015) 53–54

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

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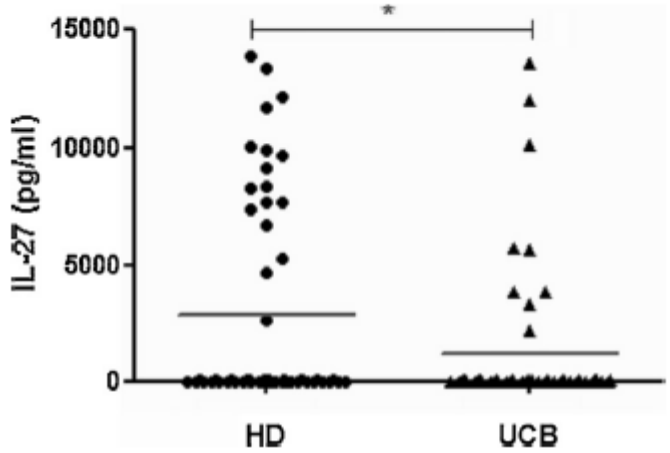

Fig. 1. The *ex vivo* human IL-27 levels (pg/ml) was determined in thawed healthy donor adult plasma (HD; $n = 52$) and neonate umbilical cord blood samples (UCB; $n = 51$) using a commercially available enzyme linked immunosorbent assay (ELISA) kit (DuoSet, R&D Systems, USA). The immunoassay was carried 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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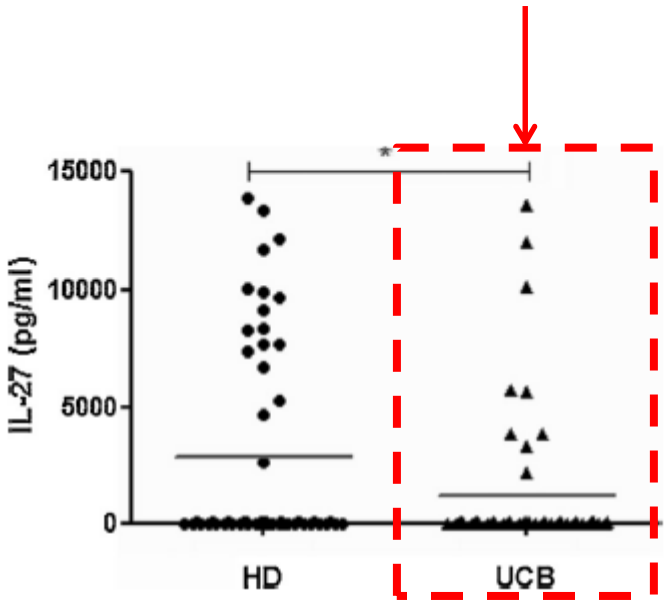

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Rational

- 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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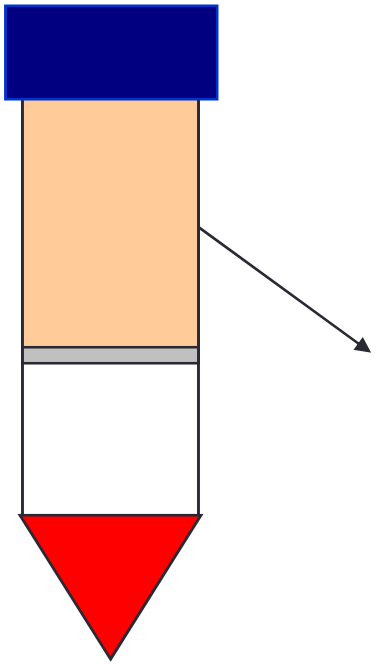

HD=28
UV=28

- 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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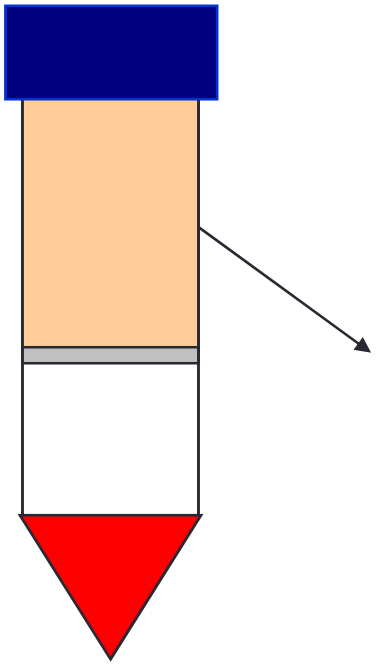

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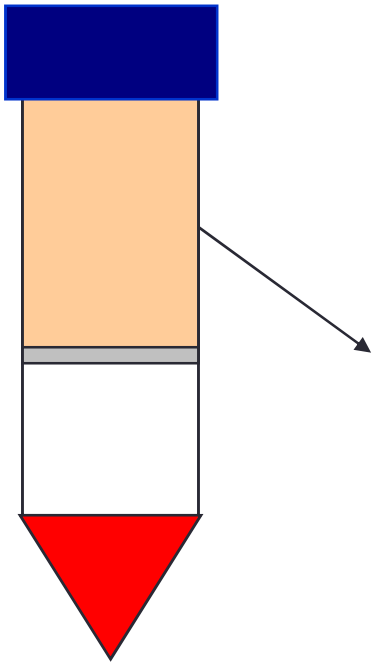

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Table 1: Characteristics of the neonate population.

Neonatal growth parameters	UV
Gestational age (weeks)	39.2 ± 0.07[a]
Birth weight (kg)	0.04 ± 0.02
Birth length (cm)	51.1 ± 0.2

Mode of delivery	UV
Induced vaginal	ND
Vaginal	2 (13%)[b]
Elective cesarean	12 (80%)
Emergency cesarean	1 (7%)[c]
NA	13 (46%)[d]

[a]Mean ± SEM; [b]Duration: 5.3h; [c]Duration: 4h; [d]IRB 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).

Cytokines	UV	HD
IL-1a	0.07 ± 0.01[a]	0.06 ± 0.01
IL-2	7.7 ± 3.2	4.6 ± 2.1
IL-4	29.4 ± 9.0	17.1 ± 7.3
IL-5	27.3 ± 4.9	24.7 ± 3.6
IL-10	77.2 ± 23.9	27.6 ± 9.5
IL-12	11.7 ± 4.2	7.9 ± 1.5
IL-13	17.9 ± 2.2	14.3 ± 0.9
IL-33	0.02 ± 0.0	0.2 ± 0.1
IFNg	69.8 ± 15.5	51.8 ± 12.0
TNFa	46.2 ± 12.8	32.4 ± 9.1
GM-CSF	22.3 ± 7.7	10.2 ± 4.0
IL-27	1.6 ± 0.5[b]	12.3 ± 3.4

[a]Mean ± SEM;

[b]p < 0.0001, when compared to HD group and based on statistical significance using the Mann-Whitney U test.

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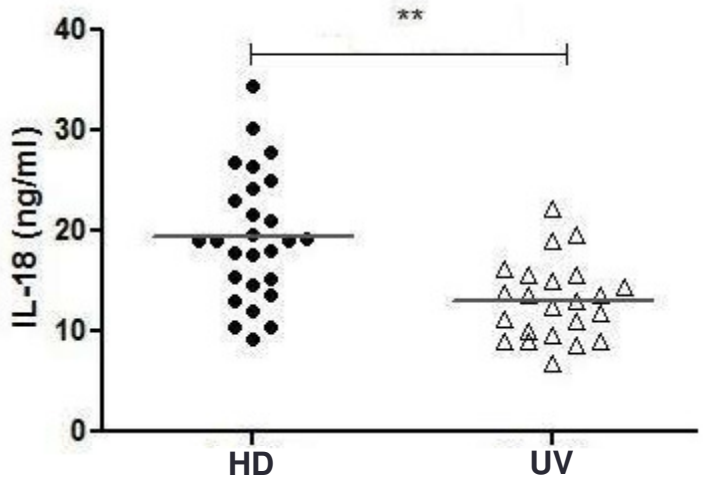
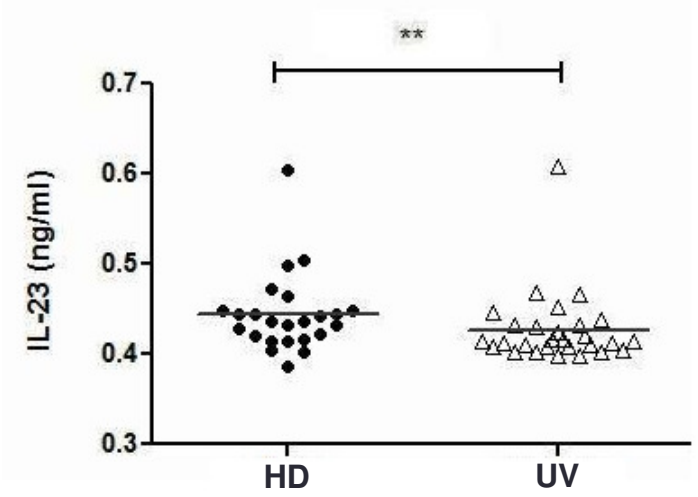
The *ex vivo* human IL-18 & IL-23 levels (ng/ml) were determined in thawed healthy donor adult plasma (HD; n=28) and umbilical cord blood samples (UV; n=28) using commercially available enzyme linked immunosorbent assay (ELISA) kits.





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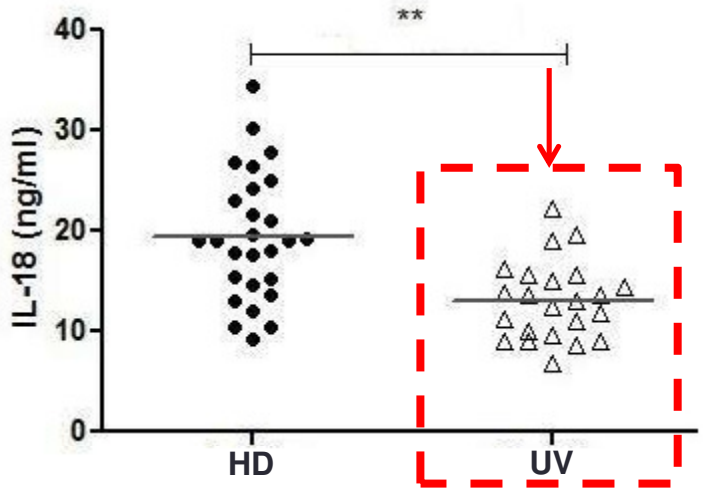

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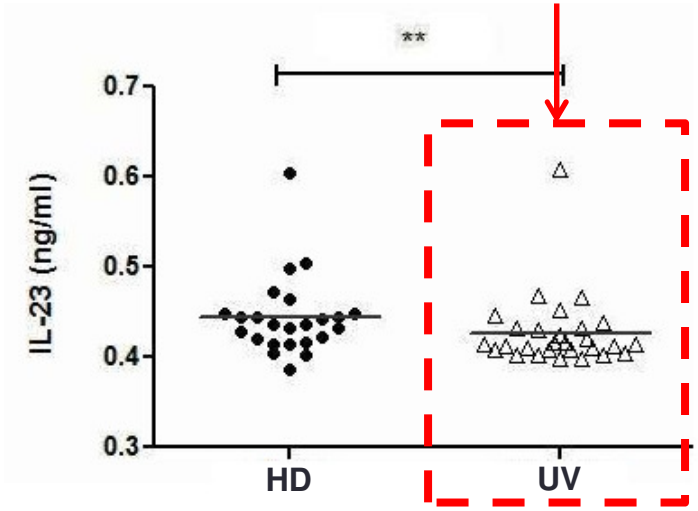

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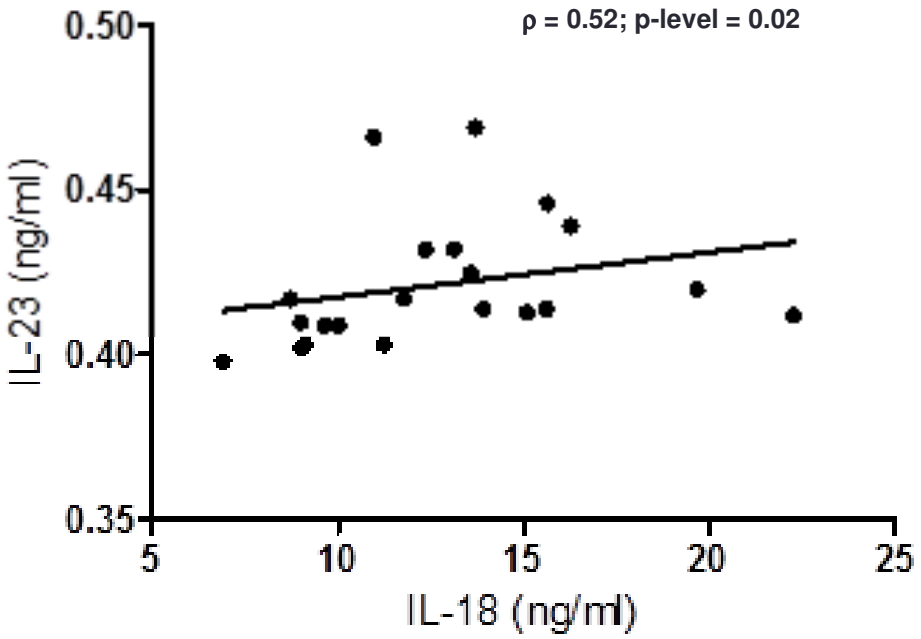

Spearman`s rank coefficient test (ρ).

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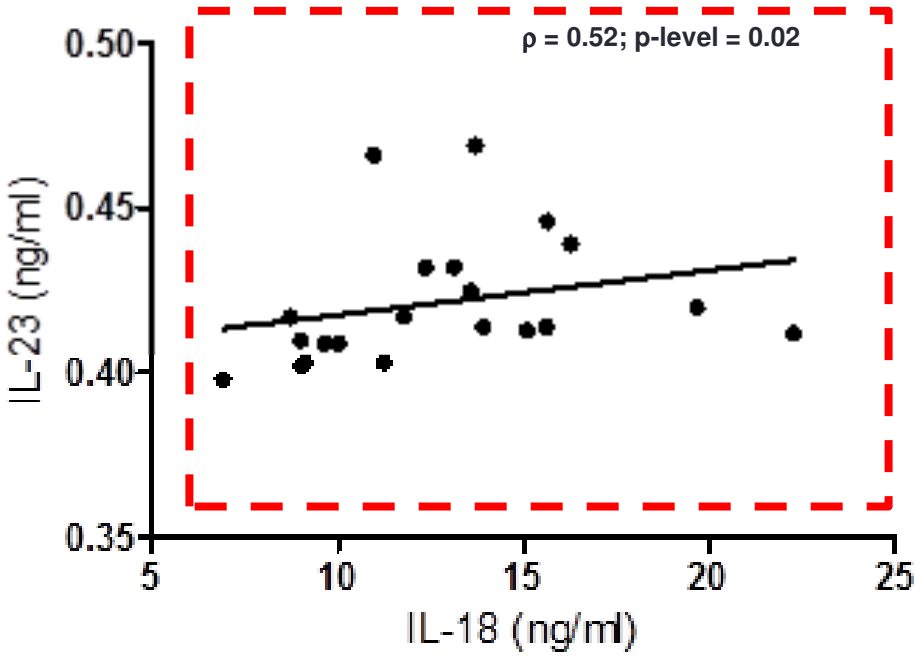

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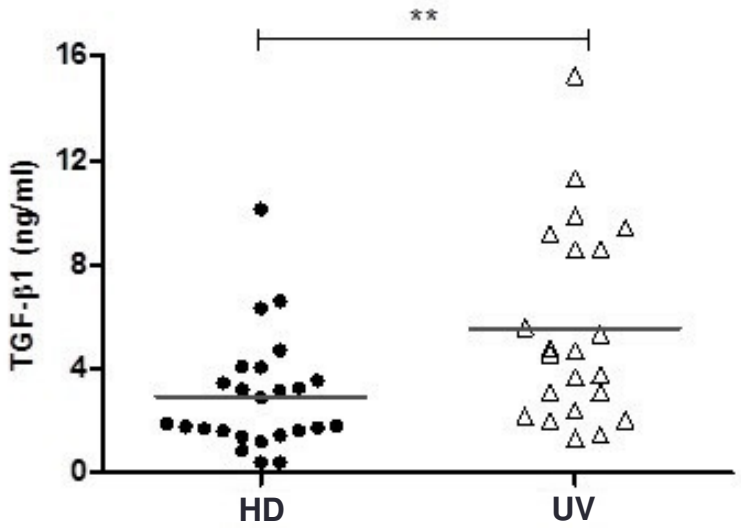

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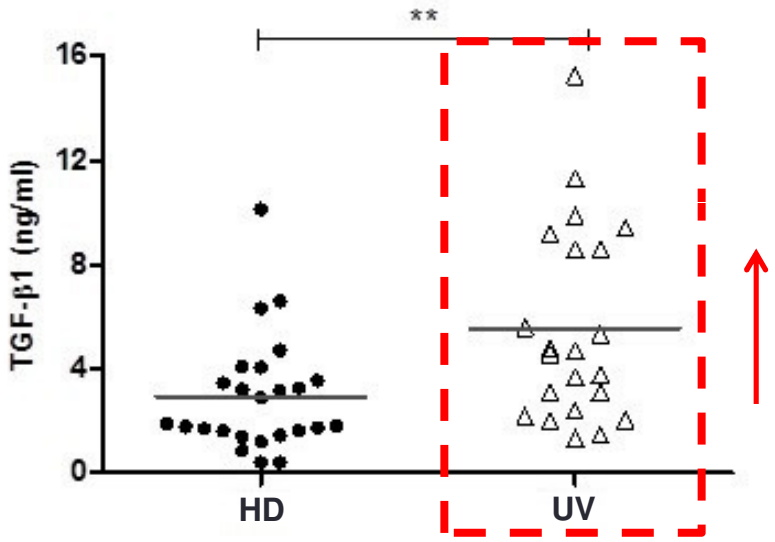

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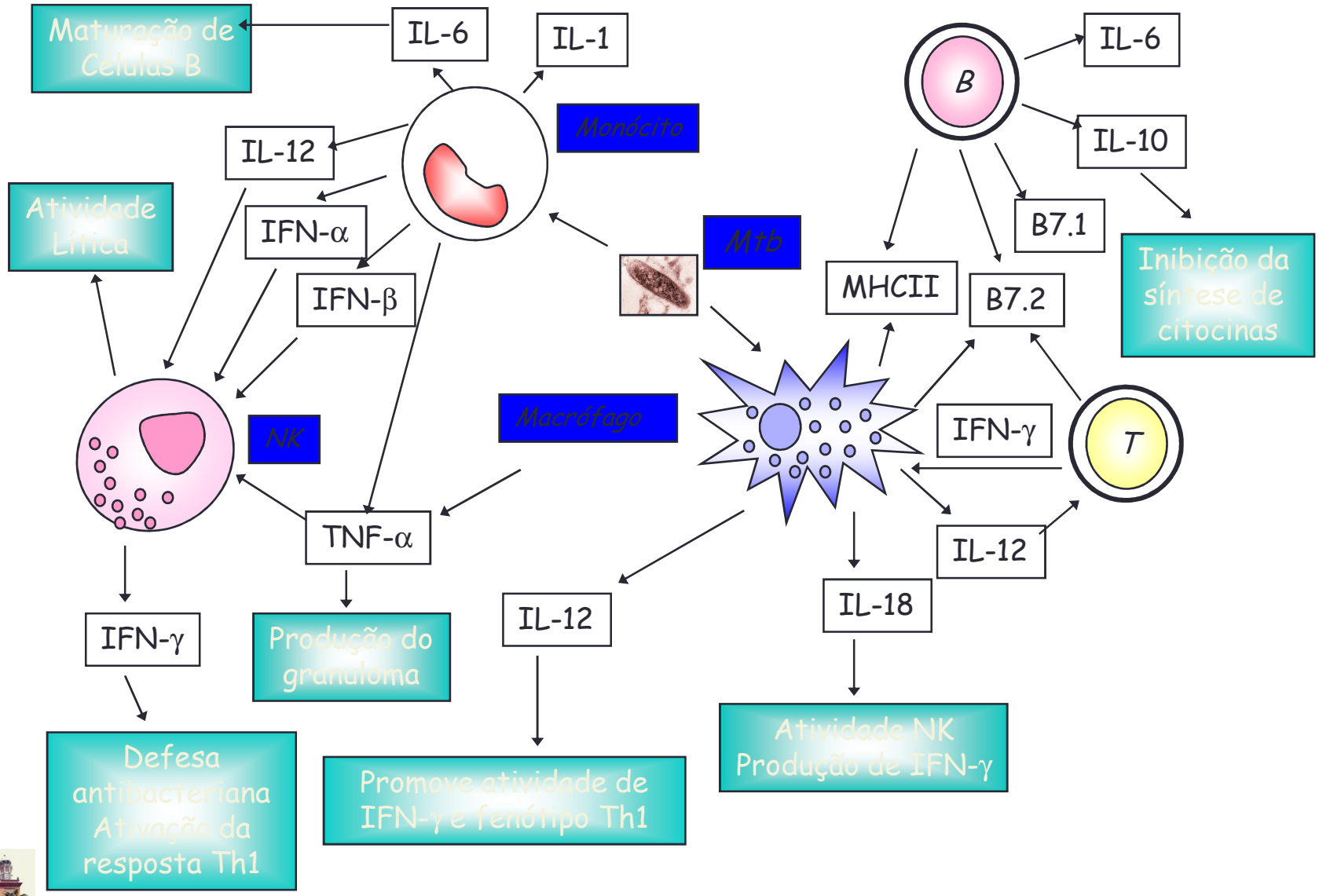
Cytokine Network



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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