

Increased cytotoxic T-cells in the airways of adults with former bronchopulmonary dysplasia

Online supplement

Appendix 2. Tables

Table E1. Perinatal characteristics of study participants

Characteristics	Healthy n=24	Asthma n=22	Preterm n=22	BPD n=22
Male/Female	12/12	9/13	10/12	11/11
Gestational age at birth, weeks	40 (37-43)	39.5 (38-42)	30 (27-32)	26.5 ^{†††} (24-31)
Birth weight, g	3458 (2670-4550)	3445 (2660-4840)	1470 (710-2200)	971 ^{†††} (583-2136)
Small for gestational age	0	0	6 (27)	8 (32)
Maternal smoking during pregnancy	5 (21)	2 (9)	3 (14)	5 (23)
Prenatal corticosteroid therapy	N/A	N/A	12 (54)	8 (36)
Caesarean section	1 (4)	1 (5)	18 (82) ***	12 (54) ***
Multiple birth	0	0	12 (55) ***	8 (36) **
Apgar score at 1 min	9 (5-9)	9 (5-10)	8 (4-10)	5 (1-9) ***
Apgar score at 5 min	10 (8-10)	10 (7-10)	9.5 (3-10) *	7 (3-10) *** [†]
Instillation of surfactant	N/A	N/A	2 (9)	7 (32)
Mechanical ventilation, days	N/A	N/A	0 (0-5)	4 (0-38) †††
CPAP, days	N/A	N/A	3 (0-19)	41 (3-70) †††
Grade BPD; mild-moderate-severe ^a (%)	N/A	N/A	N/A	7-9-6 (32-41-27)
Supplemental O ₂ , days	N/A	N/A	3 (1-26)	65 (28-167) †††
Septicemia	N/A	N/A	1 (5)	5 (23)
PDA, treated	N/A	N/A	0	7 (32) ††
ROP grade 3-4	N/A	N/A	3 (13)	5 (23)

Data are presented as median (min-max) or numbers (%). Abbreviations: BPD: bronchopulmonary dysplasia; N/A: not applicable; CPAP: continuous positive airway pressure; PDA: patent ductus arteriosus; ROP: retinopathy of prematurity. ^a: BPD diagnosis according to the definition by Jobe and Bancalari [1]. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$, comparing BPD, preterm, and asthma group to healthy control group, [†]: $P < 0.05$; ^{††}: $P < 0.01$; ^{†††}: $P < 0.001$ (BPD versus preterm).

Table E3. Three panels of monoclonal antibodies used to characterize lymphocytes in BAL.

Antigen	Function	Fluorochrome
Panel 1		
CD3	T cells	FITC
CD4	T-helper cells	Brilliant violet 421
CD8	Cytotoxic T cells	v450
CD45RO	Active T cells, Memory T cells	PE-CF594
CD45RA	Naïve/Effector T cells	PE
CD27	Degree of activation (Co-stimulatory)	PerCP-Cy5.5
CD69	Early activation	APC
Panel 2		
CD3	T cells	FITC
CD4	T-helper cells	Brilliant violet 421
CD8	Cytotoxic T cells	V450
CD56	NK cells, NKT, $\gamma\delta$ T cells	PE
CD16	NK cells, macrophages, neutrophils	APC
CD127	Memory/Effector T cells	PerCP-Cy5.5
FoxP3	Transcription factor	PE-CF594
Panel 3		
CD16	NK cells, macrophages & neutrophils	V450
CD19	B cells	Brilliant violet 421

All antibodies were purchased from BD except FOXP3 from eBioscience. Abbreviations: FITC: fluorescein isothiocyanate; PE: phycoerythrin; PerCP: Peridin Chlorophyll Protein; Cy, cyanine dye; APC, allophycocyanine

Table E3. Perinatal characteristics of study participants < gestational age 28 weeks or birth weight < 1000g

Characteristics	Preterm <i>n</i> =4	BPD <i>n</i> =18
Male/Female	2/2	10/8
Gestational age at birth, weeks	27.5 (27-28)	26 (24-29)
Birth weight, g	1049 (710-1310)	895* (583-1210)
Small for gestational age	2 (50)	6 (33)
Maternal smoking during pregnancy	1 (25)	4 (22)**
Prenatal corticosteroid therapy	2 (50)	7 (39)
Caesarean section	1 (25)	10 (55)
Multiple birth	2 (50)	6 (33)
Apgar score at 1 min	7 (6-9)	5 (1-9)
Apgar score at 5 min	7 (7-7)	7 (3-10)
Instillation of surfactant	0	8 (44)
Mechanical ventilation, days	0 (0-0)	5 (0-38)*
CPAP, days	4 (2-19)	41 (4-70)***
Grade BPD; mild-moderate-severe ^a (%)	N/A	8-6-4 (44-33-22)
Supplemental O ₂ , days	14 (1-26)	67 (144 -167)***
Septicemia	0	5 (23)
PDA, treated	0	7 (39)
ROP grade 3-4	1 (25)	5 (28)

Data are presented as median (min-max) or numbers (%). Abbreviations: BPD: bronchopulmonary dysplasia; N/A: not applicable; CPAP: continuous positive airway pressure; PDA: patent ductus arteriosus; ROP: retinopathy of prematurity.

^a: BPD diagnosis according to the definition by Jobe and Bancalari [1]. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

Table E4. Peripheral blood analyses

	Healthy	Asthma	Preterm	BPD
Number	24	22	22	22
Positive Phadiatop[®], n (%)	0	22 (100)	8 (36)	2 (9) [†]
C-reactive protein (mg/L)	0.34 (0; 2)	0.38 (0; 71)	0.34 (0.22; 0.55)	0.63 (0.21; 0.96)
α₁-antitrypsin (g/L)	1.4 (1.2; 1.7)	1.4 (1.2; 1.6)	1.3 (1.2; 1.5)	1.35 (1.1; 1.5)
Albumin (g/L)	42 (40; 44)	42.5 (40; 45)	42 (40; 44)	43 (40; 45)
Orosomucoid (g/L)	0.57 (0.54; 0.73)	0.61 (0.53; 0.75)	0.64 (0.55; 0.75)	0.62 (0.55; 0.7)
Haptoglobin (g/L)	0.88 (0.5; 1.2)	0.71 (0.42; 1.2)	0.72 (0.45; 1.1)	0.93 (0.67; 1.1)
Erythrocytes (1×10⁶/μL)	4.9 (4.4; 5.2)	4.9 (4.4; 5.0)	5.2 (4.6; 5.3)	5.1 (5; 5.3)**
Platelets (1×10⁶/μL)	256 (215; 290)	246 (220; 269)	233 (189; 264)	273 (227; 293)
Leukocytes (1×10³/μL)	5.6 (5.0; 6.5)	6.2 (5.2; 7.1)	5.7 (4.9; 6.5)	6.2 (5.7; 7.4)
Neutrophils (1×10³/μL)	3.15 (2.8; 3.9)	3.2 (2.7; 4.0)	3.4 (2.4; 3.8)	3.5 (3.1; 4.3)
Lymphocytes (1×10³/μL)	1.85 (1.7; 2.2)	1.9 (1.5; 2.1)	1.8 (1.6; 2.1)	1.9 (1.6; 2.0)
Eosinophils (1×10²/μL)	0.0 (0.0; 0.1)	0.3 (0.1; 0.3)**	0.0 (0.0; 0.1)	0.1 (0.0; 0.2)
Basophils (1×10/μL)	0.0 (0.0; 0.1)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)

Data are presented as median (IQR). Abbreviations: BPD: bronchopulmonary dysplasia; **: $P < 0.01$; comparing BPD, preterm, and asthma group to healthy control group, †: $P < 0.05$; BPD versus preterm.

Table E5. Flow cytometry of BAL lymphocytes

	Preterm vs Healthy			BPD vs Healthy			Preterm vs Asthma		
	Effect size	P value	FDR	Effect size	P value	FDR	Effect size	P value	FDR
CD4+ ^a	0.19	0.26	0.50	0.41	0.014	0.16	0.02	0.89	0.95
CD8+ ^a	0.16	0.34	0.55	0.47	0.0050	0.16	0.06	0.72	0.88
CD4+/CD8+ ^b	0.18	0.28	0.52	0.45	0.0067	0.16	0.04	0.82	0.91
CD69+CD8+ ^a	0.00	0.99	1.00	0.31	0.062	0.25	0.14	0.36	0.58
CD69+CD4+ ^a	0.40	0.014	0.16	0.10	0.59	0.76	0.19	0.22	0.46
CD27+CD45RA- ^a	0.49	0.0051	0.16	0.34	0.070	0.26	0.28	0.083	0.28
CD27+CD45RA+ ^a	0.38	0.030	0.21	0.25	0.19	0.41	0.12	0.48	0.67
CD27-CD45RA- ^a	0.03	0.87	0.94	0.20	0.29	0.53	0.17	0.30	0.53
CD27-CD45RA+ ^a	0.27	0.12	0.33	0.11	0.56	0.76	0.38	0.019	0.19
FoxP3+CD8+ ^a	0.33	0.041	0.24	0.01	0.97	1.00	0.34	0.030	0.21
FoxP3+CD4+ ^a	0.14	0.40	0.60	0.18	0.32	0.54	0.32	0.037	0.23
CD3+CD56+ ^a	0.00	1.00	1.00	0.05	0.77	0.89	0.28	0.065	0.25
CD3-CD56+ ^c	0.25	0.13	0.35	0.20	0.25	0.50	0.26	0.091	0.28
CD19+ ^d	0.24	0.18	0.40	0.11	0.58	0.76	0.05	0.76	0.89

	BPD vs Asthma			BPD vs Preterm		
	Effect size	P value	FDR	Effect size	P value	FDR
CD4+ ^a	0.30	0.060	0.25	0.22	0.18	0.40
CD8+ ^a	0.27	0.087	0.28	0.28	0.08	0.28
CD4+/CD8+ ^b	0.31	0.051	0.25	0.26	0.10	0.29
CD69+CD8+ ^a	0.26	0.10	0.29	0.40	0.011	0.16
CD69+CD4+ ^a	0.12	0.47	0.67	0.31	0.056	0.25
CD27+CD45RA- ^a	0.15	0.38	0.59	0.18	0.32	0.54
CD27+CD45RA+ ^a	0.05	0.79	0.89	0.09	0.61	0.78
CD27-CD45RA- ^a	0.15	0.39	0.59	0.05	0.79	0.89
CD27-CD45RA+ ^a	0.17	0.32	0.54	0.21	0.25	0.50
FoxP3+CD8+ ^a	0.02	0.92	0.96	0.23	0.15	0.37
FoxP3+CD4+ ^a	0.38	0.022	0.20	0.10	0.56	0.76
CD3+CD56+ ^a	0.31	0.056	0.25	0.07	0.66	0.82
CD3-CD56+ ^c	0.23	0.16	0.39	0.03	0.86	0.94
CD19+ ^d	0.06	0.74	0.89	0.15	0.42	0.61

Univariate statistics for all comparisons, along with correction for multiple testing using FDR. All comparisons with $P < 0.05$ passed FDR < 0.30 . ^a: % of CD3⁺ cells, ^b: ratio of CD3⁺ cells, ^c: % of CD3⁻ cells, ^d: % of all cells. Abbreviation: BPD: bronchopulmonary dysplasia

References

1. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *American journal of respiratory and critical care medicine* 2001; 163(7): 1723-1729.