

Title: Effects of Bacillus Calmette-Guérin (BCG) vaccination at birth on T and B lymphocyte subsets. Results from a clinical randomized trial.

Author list:

Nina Marie Birk^{1#}, Thomas Nørrelykke Nissen¹, Jesper Kjærgaard², Hans Jacob Hartling³, Lisbeth Marianne Thøstesen⁴, Poul-Erik Kofoed⁴, Lone Graff Stensballe², Andreas Andersen⁵, Ole Pryds¹, Mihai G. Netea⁶, Christine Stabell Benn⁵, Susanne Dam Nielsen³, Dorte Lisbeth Jeppesen¹.

1. Department of Pediatrics, Copenhagen University Hospital, Hvidovre, Kettegårds Allé 30, 2650 Hvidovre, 13 Denmark.

2. The Department of Pediatrics and Adolescent Medicine, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark.

3. Department of infectious diseases, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100, Copenhagen Denmark.

4. Department of Pediatrics, Kolding Hospital, Sygehusvej 24, 6000 Kolding, Denmark.

5. Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen, Denmark.

6. Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Geert Grooteplein 8, 6525 GA, Nijmegen, The Netherlands

Corresponding author: Birk, Nina Marie, MD. Department of Pediatrics, 460, Copenhagen University Hospital, 25 Hvidovre, Kettegaard Allé 30, DK-2650 Hvidovre, Denmark. Telephone: 0045 27202037, email: ninabirk@dadlnet.dk

Supplementary table A. Geometric means (GM) of lymphocyte subsets of both proportions and absolute counts assessed by flow cytometry in BCG vaccinated infants and their controls at three time points. ^a Geometric mean . ^b (cells/ μ L)

	4 days BCG (n=53) vs no BCG (n=61)				3 months BCG (n=53) vs no BCG (n=53)				13 months BCG (n=54) vs no BCG (n=52)			
	GM ^a <i>proportion (%)</i>		GM <i>cell count</i> ^b		GM <i>proportion (%)</i>		GM <i>cell count</i>		GM <i>proportion (%)</i>		GM <i>cell count</i>	
	BCG	No BCG	BCG	No BCG	BCG(n)	No BCG(n)	BCG	No BCG	BCG	No BCG	BCG	No BCG
CD 4+ T cell subsets												
<i>CD4+ T cells</i>			1815	1535			2421	2403			2215	2358
Naive cells	93	93	1647	1397	92	93	2221	2242	88	89	1987	2112
Recent thymic emmigrants	59	57	1039	858	62	60	1489	1437	62	62	1393	1453
Tregs	8.4	8.0	154	121	7.3	7.3	162	174	7.7	7.5	173	176
Th 17 cells	0.7	0.6	12	8.9	1.6	1.3	35	33	2.3	2.2	52	53
Central memory cells	4.4	3.8	74	58	4.5	3.7	110	90	8.2	6.1	182	145
Effector memory cells	0.9	0.7	14	11	1.0	0.6	24	15	0.8	0.9	18	20
Late differentiated cells	0.03	0.03	0.6	0.5	0.1	0.1	2.9	2.7	0.1	0.2	2.4	4.0
Chronic activated cells	1.0	0.8	18	13	1.0	1.2	23	27	1.1	1.3	25	31
Apoptotic cells	0.2	0.2	3.5	2.9	0.3	0.3	7.2	8	0.3	0.5	6.8	13
CD8+ T cell subsets												
<i>CD8+ T cells</i>			502	464			687	761			789	884
Naive cells	94	95	458	434	84	86	576	652	80	76	631	678
Recent thymic emmigrants	96	97	469	443	90	90	618	682	86	83	678	748
Tc 17 cells	1.6	1.7	7.2	7.8	0.9	1.0	5.8	7.9	0.8	0.8	6.7	7.2
Central memory cells	0.8	0.8	3.7	3.8	0.9	0.8	6.0	6.0	1.4	1.1	11	10
Effector memory cells	0.3	0.3	1.2	1.3	1.0	0.6	6.8	4.8	1.3	1.4	10	12
Late differentiated cells	0.9	1.0	4.3	4.8	0.8	0.8	5.2	6.2	0.9	1.5	6.9	13
Chronic activated cells	1.6	1.5	8.5	6.8	2.5	3.1	17	24	2.6	2.9	20	26
Apoptotic cells	4.0	4.0	20	18	6	6.4	41	49	11	14	91	120
CD 19+ B cell subsets												
<i>CD19+ B cells</i>			391	365			1230	1213			1342	1340
CD19+CD24 ^{high} +CD38 ^{high}	77	78	302	279	35	36	428	438	19	18	251	242

Supplementary table B. Background characteristics for infants randomized to BCG or no intervention in The Danish Calmette Study. Comparing infants included in the substudy on T and B cell subsets with infants who were randomized at Copenhagen University Hospital, Hvidovre in the inclusion period, but who were not included into the substudy.

	Substudy on T and B cells	Randomized at HvH	p value
Enrollment	n=118	n=483	
Age at randomization ^a			
0-1 days	73 (62%)	250 (52%)	0.05
2-7 days	45 (38%)	233 (48%)	
Male sex ^a	61 (52 %)	237 (49 %)	0.30
Gestational age (weeks) ^b	40 (38-41)	40 (38-41)	0.33
Weight (Kg) ^b	3.5 (2.9-4.0)	3.5 (2.8-4.0)	0.54
Caesarean section ^a	22 (19 %)	121 (25 %)	0.14
Maternal BCG ^a	22 (19%)	93 (20%) [6]	0.87
Parental ethnicity different from Danish ^a	19 (16%)[1]	116 (24%)	0.07
Maternal smoking during pregnancy ^a	8 (7%) [1]	53 (11%)	0.17
Level of maternal education ^a	[1]		0.56
Basic schooling and non-theoretical education	25 (21%)	112 (23%)	
Theoretical education incl BA level	53 (45%)	223 (46%)	
Master level or more	39 (34%)	148 (31%)	
Siblings ^a	40 (34%)	232 (39%)	0.24
Atopic predisposition ^c	78 (66%)	289 (60%)	0.21

^a N number (Frequency) [not available],

^b Median (10-90 percentiles)[not available]

^c Atopic disposition is defined as at least one first degree relative with atopic disease. Atopic disease is defined as physician-diagnosed atopic eczema, asthma, allergic rhino-conjunctivitis or food allergy