

European Journal of Immunology

Supporting Information

for

DOI 10.1002/eji.201746971

Theo van den Broek, Asaf Madi, Eveline M. Delemarre, Alvin W. L. Schadenberg, Kiki Tesselaar, José A. M. Borghans, Stefan Nierkens, Frank A. Redegeld, Henny G. Otten, Maura Rossetti, Salvatore Albani, Rachel Sorek, Irun R. Cohen, Nicolaas J. G. Jansen and Femke van Wijk

Human neonatal thymectomy induces altered B-cell responses and autoreactivity

Supplemental information:

Human neonatal thymectomy induces altered B-cell responses and auto-reactivity

Theo van den Broek, Asaf Madi[#], Eveline M. Delemarre[#], Alvin W.L. Schadenberg, Kiki Tesselaar,

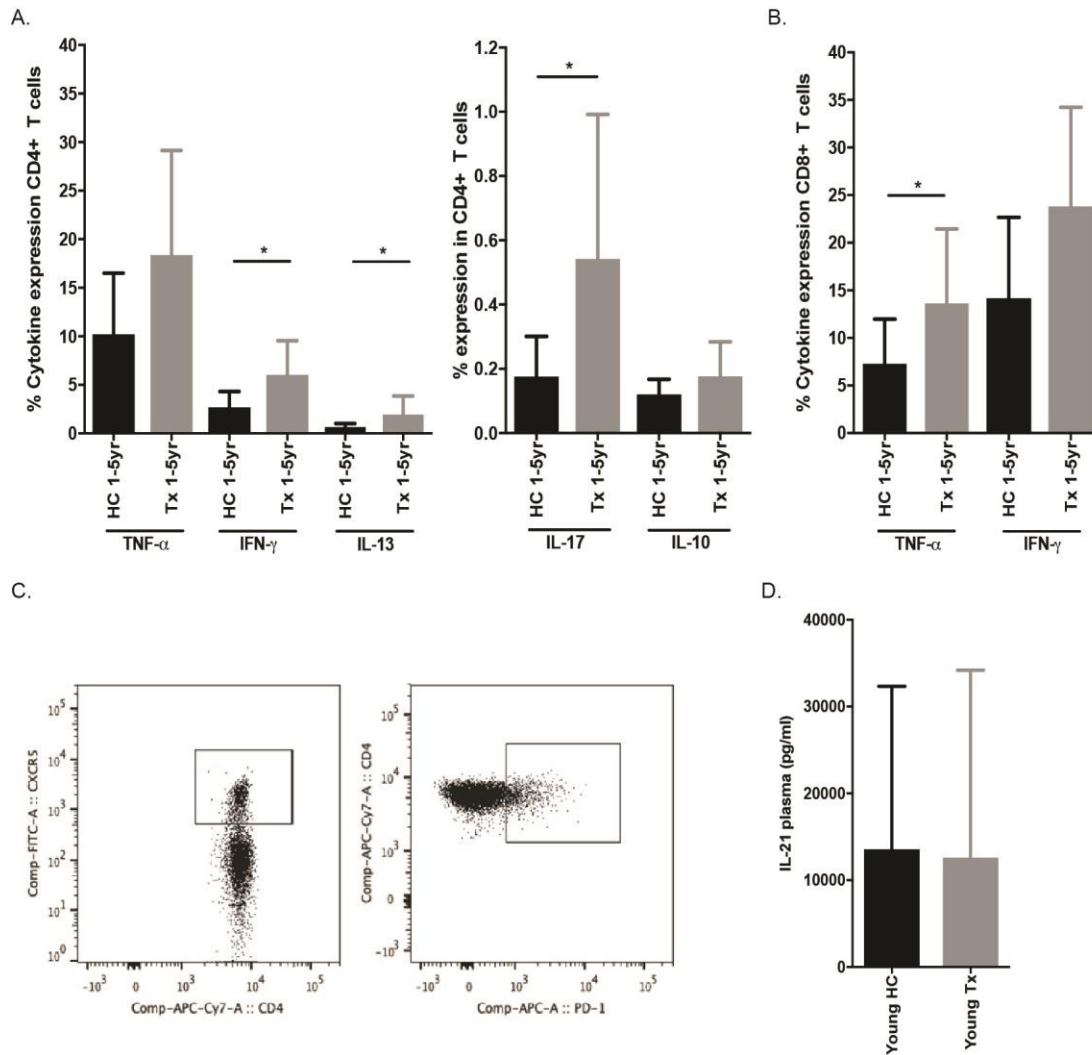
José A.M. Borghans, Stefan Nierkens, Frank A. Redegeld, Henny G. Otten, Maura Rosetti,

Salvatore Albani, Rachel Sorek, Irun R. Cohen, Nicolaas J.G. Jansen, Femke van Wijk

authors contributed equally

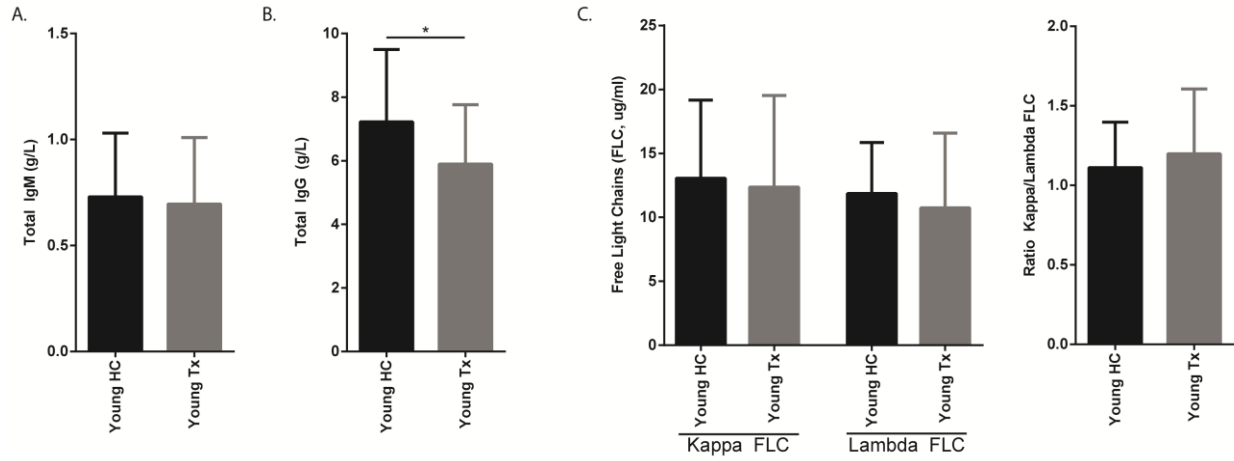
Patient	Age at thymectomy (days)	Age (post-thymectomy; months)	% Memory CD4+ T cells
1	7	15	28,7
2	7	19	34,8
3	11	12	42,6
		23	50,6
4	21	23	37,9
5	10	24	41,6
6	9	25	28,5
7	7	27	48,3
8	5	24	72,2
9	30	32	53,2
		69	65,9
10	7	25	23,5
11	3	35	63,7
12	18	37	72,5
13	n/a	36	10,2
14	n/a	25	25,2
15	n/a	31	13,1
16	n/a	47	22,7
17	n/a	28	9,9
18	n/a	52	20,3
19	n/a	14	9,5
20	n/a	15	9,9
21	n/a	33	24,2

Supplemental Table 1. Overview of thymectomized patients (young Tx) and healthy controls (young HC) regarding age and proportion of memory CD4+ T cells (CD3+CD4+ CD45RO+). Patients 1-10 are young Tx, patients 11-19 are young HC.



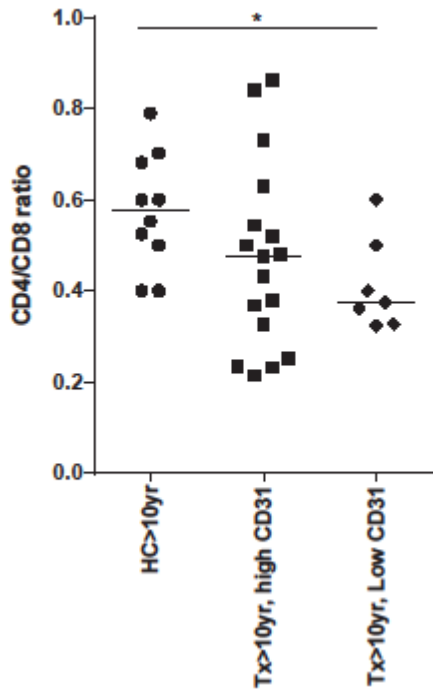
Supplemental figure 1. Neonatal thymectomy results in a pro-inflammatory cytokine expression profile.

a) Cytokine expression of CD4+ T cells in healthy control (young HC, n=9) and thymectomized (young Tx, n=11) children, b) Cytokine expression of CD8+ T cells in healthy control (young HC, n=9) and thymectomized (young Tx, n=11) children, c) Representative FACS gating strategy for CD4+ CXCR5+PD-1+ Tfh cells, d) Plasma IL-21 levels (pg/ml) of healthy control (young HC, n=19) and thymectomized (young Tx, n=11) children. Mean (and SD) depicted in bar graphs. * for <0.05 via Mann-Whitney U-test

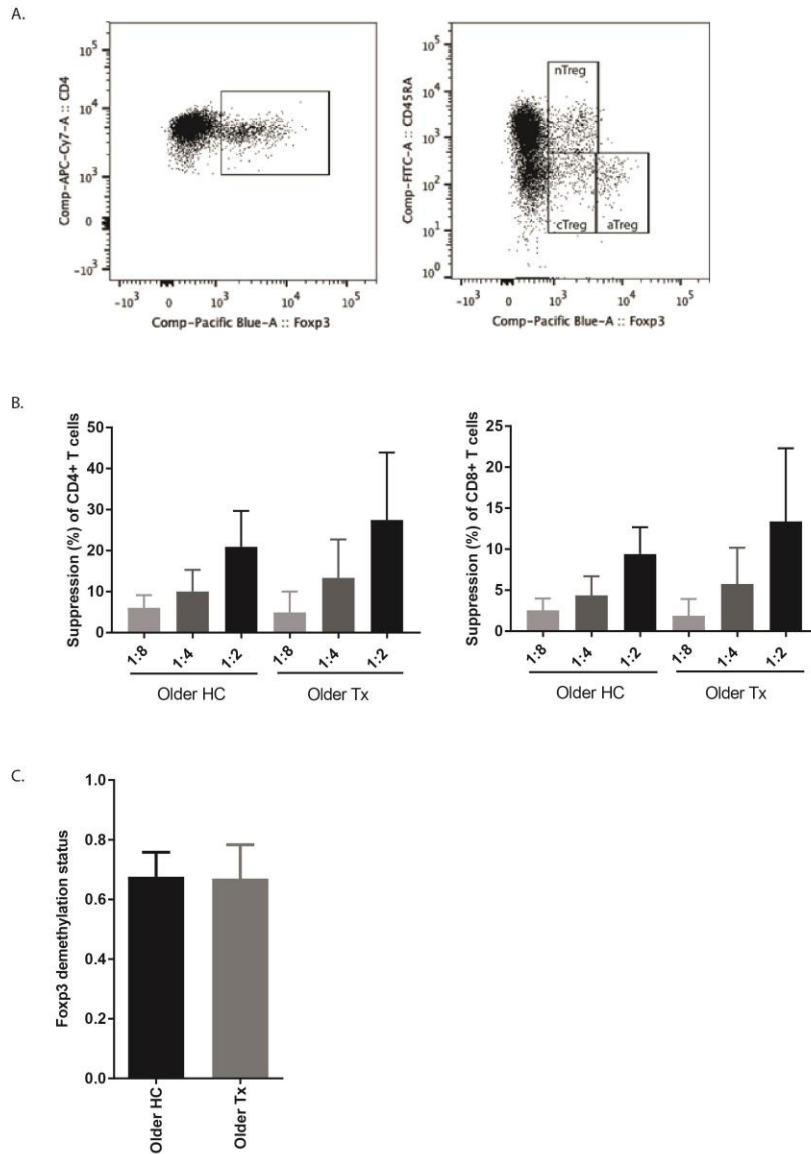


Supplemental figure 2. Immunoglobulin production is relatively unaltered after neonatal thymectomy

a) Total IgM (g/L) in plasma of HC (n=31) and Tx (n=25) b) Total IgG (g/L) in plasma of HC (n=31) and Tx (n=25) c) left panel, amount of kappa and lambda free light chain in HC (n=12) and Tx (n=20). right panel ratio of Kappa/lambda FLC for HC and Tx. * for P<0.05, mean and SD shown



Supplemental figure 3. CD4/CD8 ratio of older HC (n=10), older Tx, high CD31 (n=17), and older Tx, low CD31 (n=7), median shown.



Supplemental figure 4. Regulatory T cell phenotype, function and stability.

a) Representative gating for Treg (Foxp3+) cells (left panel) and for each Treg population according to Foxp3 and CD45RA expression (right panel). Both dotplots were initially gated on live cells within lymphocyte gate, followed by single cell selection and CD3+ CD4+ T cell gating.

b) Percent suppression of CD4+ T cells (left panel) and CD8+ T cells (right panel) by regulatory T cells from older HC and older Tx in a ratio of 1:2, 1:4 and 1:8 (Treg : Tcell), older HC n=5 for all ratios, older Tx n=5 for ratio 1:4 & 1:8, n=4 for 1:2. c) Demethylation status of the Treg specific demethylation region (TSDR) of older HC (n=13) and older Tx (n=16). Mean with SD shown