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Data in Brief

Transcriptional profiling of intestinal CD4$^+$ T cells in the neonatal and adult mice

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ABSTRACT

The adult small intestine contains more than half of the body's lymphocytes in order to maintain homeostasis with the commensal microbiota. Birth marks a transition of the intestine from a sterile to an increasingly colonized environment. The data described in this article are incremented into the work published by Torow et al. titled "Active suppression of intestinal CD4$^+$TCRαβ$^+$ T lymphocyte maturation during the postnatal period" [1]. While most of the CD4 T cells found in the adult small intestine have an activated phenotype marked by expression of helper lineage specific genes neonatal lymphocytes exhibit a naïve phenotype. Further, direct comparison of neonatal CD4 T cells from the small intestine and the gut draining mesenteric lymph node (mLN) reveals a global transcriptional 'inactivity' of the small intestinal CD4 T cells. Here, we describe in more detail the experimental design, sample preparation and analysis that were performed to obtain and interpret the microarray data. The data set is publicly available through the Gene Expression Omnibus (GEO) database with accession number GSE60515, and the analysis and interpretation of these data are included in Torow et al. [1]

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Specifications	
Organism/cell line/tissue	*Mus musculus*
Sex	Male and female
Microarray type	026655AsQuadruplicatesOn4x180k (Agilent Technologies; AMADID 048306)
Data format	Raw and processed
Experimental factors	Primary CD4 T cells from small intestinal tissue of neonatal and adult mice
Experimental features	Transcriptional profile of CD4 T cells from total small intestine of neonatal mice was compared to those of CD4 T cells from adult lamina propria or Peyer's patches as well as mLN and thymus.
Consent	NA
Sample source location	NA

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE60515.

2. Experimental design, materials and methods

2.1. Experimental design

CD4 T cells were extracted from C57Bl/6N mice. Age, organ and the number of mice pooled per sample are indicated in Table 1. One litter of neonatal mice consisted of 6–12 animals. Male and female mice were used randomly throughout the study. The array was performed in quadruplicates.

2.2. Cell isolation and FACS sorting

Total small intestinal leukocytes from neonatal tissue were isolated as previously described [2]. For that, small intestines were excised, mesenteric fat was carefully removed and the organs were opened longitudinally. The contents were carefully removed and the remaining tissue was digested in Liberase™/DNaseI/RPMI/10% fetal calf serum (FCS) (30 μg/ml Liberase, 40 U/ml DNase, Roche) at 37 °C for 45 min. For the isolation of adult *lamina propria* lymphocytes the tissue was digested twice following the above protocol. After the enzymatic treatment the tubes were shaken vigorously filtered, and viable leukocytes were purified on a discontinuous 40%/70% Percoll™ (GE Healthcare) gradient. Peyer's patch leukocytes were obtained by carefully excising Peyer's

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Table 1
FACS sorted CD4 T cells from following groups were included in the microarray analysis. SI, small intestine; Th, thymus; mLN, mesenteric lymph node; LP, *lamina propria*; PP, Peyer's patch.

Age (day after birth)	Organ	# of mice/sample	# of samples
d6	SI	1 litter	4
d6	Th	1 litter	4
d6	mLN	1 litter	4
d11	SI	1 litter	4
d28	LP	2–3	4
d56	LP	2–3	4
d56	PP	2–3	4
d56	mLN	2–3	4

patches and straining them through a mesh. Thymocytes were obtained by straining thymi through a mesh. Cell suspensions were subsequently subjected to FACS sorting. Sorting was performed on FACS aria from BD. All samples were pregated on $CD45^+$ live singlets and sorted for $TCRb^+ CD4^+ CD8\alpha^-$ cells. Sorted cells were subjected to post-sort analysis for quality control (Fig. 1).

2.3. RNA preparation, quantification and quality control

RNA was extracted from $0.5–2 * 10^5$ FACS sorted CD4 T cells using the RNeasy Micro Kit (Qiagen). The manufacturer's protocol for 10^5 cells was followed and a DNase digestion step was included.

Total RNA yields were quantified by both, photometric measurements (Nanodrop-1000, PeqLab) and by the use of the RNA 6000 Pico Kit assay (Agilent Bioanalyzer 2100). The latter assay was also used to assess RNA integrity. RNA integrity numbers (RINs) are listed in Table 2. No systematic correlation between RIN values and class affiliation could be observed. Retrieved total RNA yields ranged from 4 to 238 ng (Table 2).

2.4. Sample processing

4–8 ng of total RNA (based on Bioanalyzer quantification results) was used to prepare aminoallyl-UTP-modified (aaUTP-) cRNA (Amino Allyl MessageAmp™ II Kit; #AM1753; Life Technologies) as recommended by the company (applying one round of amplification). cDNA and cRNA yields for each sample are listed in Table 2. Prior to the reverse transcription reaction, 1 μl of a 1:50,000 dilution of Agilent's 'One-Color spike-in Kit stock solution' (#5188-5282, Agilent Technologies) was added to each total RNA sample. The labeling of aaUTP-cRNA was performed by the use of Alexa Fluor 555 Reactive Dye (#A32756; Life Technologies). Yield of labeled cRNA is listed in Table 2. Relative fluorescence incorporation was determined by calculating the ratio of absorption at 556 nm and 259 nm (Table 2).

2.5. Hybridization, washing and scanning of microarrays

cRNA fragmentation, hybridization and washing steps were carried out as recommended in the 'One-Color Microarray-Based Gene Expression Analysis Protocol V5.7', except that 34–45 ng of each fluorescently labeled cRNA population was used for hybridization (Table 2). Quantification of labeled cRNA samples before hybridization was performed with the RNA 6000 Pico Kit assay (Agilent Bioanalyzer 2100). Slides were scanned on the Agilent Micro Array Scanner G2565CA (pixel resolution 3 μm, bit depth 20).

2.6. Microarray type used

The microarray study was performed using a refined version of the Whole Mouse Genome Oligo Microarray 4x44k v2 (Design ID 026655, Agilent Technologies), called '026655AsQuadruplicatesOn4x180k' (Design ID 048306) developed at the Research Core Unit Transcriptomics of Hannover Medical School. Microarray design was defined at Agilent's eArray portal using a 4x180k design format for mRNA expression as template. All non-control probes of design ID 026655 were selected to be printed four times onto one 180 k Microarray (on-chip quadruplicates). Control probes required for proper Feature Extraction software algorithms were determined and placed automatically by eArray using recommended default settings.

2.7. Data extraction and processing

Data extraction was performed with the 'Feature Extraction Software V10.7.3.1' using the recommended extraction protocol file 'GE1_107_Sep09.xml'. Data were further processed using Omics Explorer software v3.0 (Qlucore). For that, extracted raw data were imported under default import settings for Agilent One Color mRNA Microarrays. Accordingly, data processing steps were: 1) removal of control measurements, 2) log base 2 transformation, 3) normalization of non-

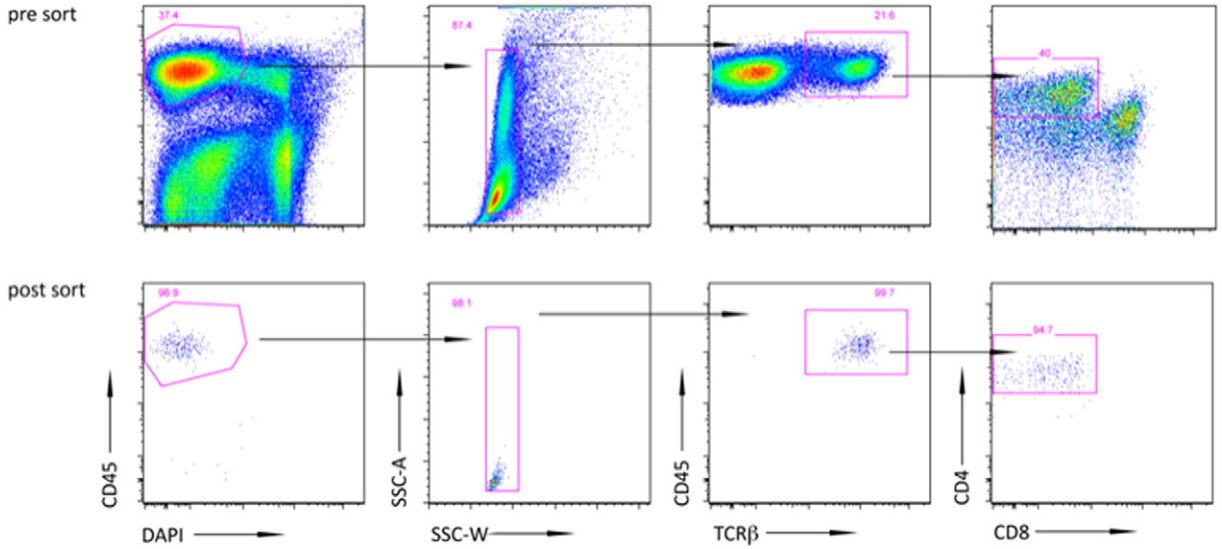

Fig. 1. Gating strategy for FACS sorting of CD4 T cells applied throughout this study.

Table 2
Characteristics and yields of nucleic acid samples and intermediates generated and used in the study. M #, specific Microarray Identifier; rep, replicate; d, day after birth; SI, small intestine; Th, thymus; mLN, mesenteric lymph node; PP, Peyer's patch; LP, *lamina propria*; RIN, RNA integrity number; cRNAlab, fluorescently labeled cRNA.

M #	Sample description	Total RNA yield based on Bioanalyzer Pico chip (ng)	RIN	Used amount of total RNA for cDNA-synthesis (ng)	cDNA yield (μl)	Used amount of cDNA for cRNA-synthesis (μl)	cRNA yield based on Nanodrop (ng)	Used amount of cRNA for labeling (ng)	cRNAlab yield based on Bioanalyzer Pico chip (ng)	Relative fluorescence incorporation to cRNAlab (OD 556/OD 259)	Used amount of cRNAlab for hybridization (ng)	Hybridization date
M4182	d6 SI_rep1	23	7.6	4	14; 14[a]	14; 14[a]	467; 382[a]	152	81	0.535	45	22-Aug-2013
M4183	d6 Th_rep1	16	7.8	5	14	14	614	189	103	0.625	45	22-Aug-2013
M4184	d11 SI_rep1	10	8.0	4	14	14	121	121	43	0.700	43	22-Aug-2013
M4186	d6 SI_rep2	39	7.8	5	14	14	448	128	47	0.655	45	22-Aug-2013
M4187	d6 Th_rep2	12	9.4	4	14	14	417	141	48	0.692	45	22-Aug-2013
M4188	d11 SI_rep2	26	7.3	4	14; 14[a]	14; 14[a]	52; 85[a]	137	138	0.710	45	22-Aug-2013
M4190	d6 SI_rep3	4	7.5	4	14	14	602	100	55	0.543	45	26-Aug-2013
M4191	d6 Th_rep3	7	9.6	4	14	14	739	187	71	0.688	45	26-Aug-2013
M4192	d11 SI_rep3	238	6.6	4	14	14	43	43	66	0.481	45	26-Aug-2013
M4194	d6 SI_rep4	119	7.0	4	14	14	45	45	59	0.391	45	26-Aug-2013
M4195	d6 Th_rep4	32	7.6	4	14	14	83	83	121	0.538	45	26-Aug-2013
M4196[b]	d11 SI_rep4	34	8.7	28	14	14	290	145	214	0.644	45	26-Aug-2013
M4338	d6 mLN_rep1	24	9.1	4	14	14	86	86	104	0.500	45	11-Dec-2013
M4339	d28 LP_rep1	74	7.3	8	14	14	64	64	39	0.550	39	11-Dec-2013
M4340	d56 mLN_rep1	7	7.6	4	14	14	125	125	102	0.828	45	11-Dec-2013
M4341	d56 PP_rep1	9	9.2	4	14	14	495	495	94	0.593	45	11-Dec-2013
M4342	d56 LP_rep1	12	7.9	8	14	14	146	146	98	0.731	45	11-Dec-2013
M4343	d6 mLN_rep2	24	7.9	4	14	14	304	304	340	0.709	45	11-Dec-2013
M4344	d28 LP_rep2	62	7.6	8	14	14	96	96	136	0.690	45	11-Dec-2013
M4345	d56 mLN_rep2	32	7.3	8	14	14	167	167	230	0.609	45	11-Dec-2013
M4346	d56 PP_rep2	14	8.9	8	14	14	179	179	261	0.736	45	11-Dec-2013
M4347	d56 LP_rep2	53	7.6	8	14	14	223	223	156	0.816	45	11-Dec-2013
M4348	d6 mLN_rep3	67	8.5	4	14	14	511	511	44	0.750	44	11-Dec-2013
M4349	d28 LP_rep3	38	8.6	8	14	14	135	135	172	0.612	45	11-Dec-2013
M4350	d56 mLN_rep3	31	7.8	8	14	14	616	616	59	0.750	45	11-Dec-2013
M4351	d56 PP_rep3	20	8.1	4	14	14	433	433	34	0.444	34	11-Dec-2013
M4352	d56 LP_rep3	4	8.1	4	14	14	94	94	56	0.684	45	11-Dec-2013
M4353	d6 mLN_rep4	65	7.3	8	14	14	98	98	87	0.385	45	11-Dec-2013
M4354	d28 LP_rep4	40	8.5	8	14	14	138	138	133	0.605	45	11-Dec-2013
M4355	d56 mLN_rep4	31	8.2	8	14	14	161	161	229	0.712	45	11-Dec-2013
M4356	d56 PP_rep4	7	8.1	4	14	14	122	122	53	0.609	45	11-Dec-2013
M4357	d56 LP_rep4	49	8.2	8	14	14	456	456	41	0.667	41	11-Dec-2013

[a] First round of cDNA/cRNA synthesis was not successful in terms of quality or yield and was repeated.
[b] The cRNA population that gave rise to M4196 showed an elevated fragment length distribution and an elevated yield compared to the rest of the study. After several unsuccessful attempts to generate sufficient amounts of cRNA from 4 ng of total RNA, utilized input amount was increased to 28 ng for this sample to enable the generation of sufficient amounts of cRNA for labeling and hybridization.

control values by shifting to 75 percentile, 4) averaging of values from on-chip replicates, and 5) baseline transformation to the median.

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