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SUPPLEMENTAL MATERIALS AND METHODS

H-CDR3 spectratyping

Total RNA from $4 \times 10^{4-5}$ sorted cells was isolated with the RNeasy Micro Kit (QIAGEN) and reverse transcribed by random priming with the ProSTAR First Strand Reverse Transcription-PCR Kit (Stratagene). The volume of cDNA was then adjusted to achieve an equivalent number of cells per microliter, depending on the initial number of cells in each sorted fraction. For H-CDR3 size spectratyping, V_H5.51, V_H6, V_H3.15, V_H3.30/33, and V_H3.23 μ and γ transcripts were amplified with a seminested strategy. In a first step and for each V_H, three independent PCR amplifications were performed on cDNA aliquots (each being equivalent to ~4,000 cells) with Pfu turbo polymerase (Stratagene), using forward V_H-leader–specific primers with the appropriate reverse primers specific for the C_H1 region of the μ (μC_H1) or the γ heavy chain (γCH1).

Amplification was performed as follows: 20 cycles of 45 s at 94°C, 1 min at 62°C, and 1 min 30 s at 72°C. The PCR products were then pooled, purified with the NucleoSpin Extract II kit (Macherey-Nagel), and subjected to seminested PCR (in triplicate) with Phusion DNA polymerase for 20 cycles (10 s at 98°C, 20 s at 60°C, and 20 s at 72°C) using the same μC_H1 or γC_H1 primers with internal V_H-FR1 primers. PCR products of the expected size (~400 bp) were gel purified and fluorescently labeled using a run-off procedure, and, in some cases, aliquots were size separated on a polyacrylamide gel when sequencing was required (see below). The run-off reaction consisted of 10 cycles of 30 s at 94°C, 30 s at 58°C and 1 min at 72°C, with Taq DNA polymerase (Biolabs) and a specific V_H-FR3 primer labeled 5′ with the fluorescent dye HEX. The run-off products were subjected to electrophoresis on an ABI PRISM 3100 genetic analyzer, in the presence of the GeneScan 400HD ROX Size Standard and were analyzed with Genemapper version 4 (both from Applied Biosystems). The different spectratypes shown in Fig. 1, Fig. 2 and Fig. S1 were thus performed using a cDNA volume equivalent to at least 10–12,000 B cells, but similar profiles were obtained when using an equivalent of 5–6,000 B cells (not depicted). Moreover, a spectratyping of the V_H5.51 gene performed on either DNA or RNA, extracted with the AllPrep DNA/RNA Mini Kit (QIAGEN) from the same pool of 10^5 cells (either IgD^+CD27^+, IgD^-CD27^+, or naive cells) gave similar results, thus excluding a bias in analysis that could be introduced by the presence of $CD20^+$ plasmablasts. For each spectratype, the Genemapper software provided tables of size (base pair) and peak heights, with peak height proportional to the total number of H-CDR3 fragments of a given size. Considering only peaks above a chosen cutoff point (≥2.5% of maximal peak intensity for a given experiment), mean peak size was equal to the sum of [size × heights] divided by the total of peak heights. Taking into account the localization of the μC_H1, γC_H1, and FR3 primers, mean HCDR3 size can be inferred from mean peak size.

In parallel, for intrapeak sequence analysis, aliquots of the V_H-FR1 μ/γ nested PCR were size-fractionated on a 5% polyacrylamide-7 M urea gel and visualized by silver staining with Silver Sequence DNA Staining reagents (Promega). Individual bands of interest (corresponding to a given CDR3 length) were excised from the gel, reamplified (same conditions as for the seminested PCR), and sequenced, to evaluate the clonal diversity of each peak.

The following primers (Sigma-Aldrich) were used for H-CDR3 spectratyping: V_H6-Leader, 5′-GTCTGTCTCCTTCCTCATCTTCC-3 ; V_H3.15-Leader, 5′-CTGAGCTGGATTTTCCTTGC-3′; V_H3.23-Leader, 5′-CTGAGCTGGCTTTTTCTTGTG-3′; V_H3.30/33-Leader, 5′-GTTTTCCTCGTTGCTCTTTTAAG-3′; V_H5.51-Leader, 5′-GGTCAACCGCCATCCTCGC-3′; V_H6-FR1, 5′-CCTCGCAGACCCTCTCAC-3′; V_H3.15-FR1, 5′-TTGGTAAAGCCTGGGGGGTC-3′; V_H3.23-FR1, 5′-AGTGTGAGGTG-CAGCTGTTG-3′; V_H3.30/33-FR1, 5′-GGCGTGGTCCAGCCTGGGA-3′; V_H5.51-FR1, 5′-GGTGAAAAAGCCCGGGGAG-3′; V_H6-FR3 HEX, 5′-TGTGACTCCCGAGGACACG-3′ ; V_H3.15-FR3 HEX, 5′-CACAGCCGTGTATTACTGTAC-3′; V_H3.23-FR3 HEX, 5′-CACGGCCGTATATTACTGTGC-3′; V_H3.30/33-FR3 HEX, 5′-ACACGGCTGTGTATTACTGTG-3′; V_H5.51-FR3 HEX, 5′-AGGCCTCGGACACCGCCATG-3′; μC_H1, 5′-AAAAGGGTTGGGGCGGATGCAC-3′; γCH1, 5′-AAGACCGATGGGCCCTTGGTGG-3′.

The J_H4-J_H5 intronic region was amplified with a mixture of 6 FR3 primers: FR3#1, 5′-GACACGGCTGTGTAT-TACTGTGC-3′ FR3#2, 5′-GACACGGCCGTGTATTACTGTGC-3′ FR3#3 5′-GACACCGCCATGTATTACT-GTGC-3′ FR3#4, 5′-GACACAGCCACATATTACTGTGC-3′ FR3#5, 5′-GACACAGCCGTGTATTACTGTAC-3′ FR3#6, 5′-GACACGGCCTTGTATTACTGTGC-3′ mixed in a 5:7:1:1:1:1 ratio), and a primer binding 5′ to the J_H5 exon: iJH4-5, 5′-GTCGAACCAGTTGTCACATTGTG-3′.

Table S1. Intrapeak clonal diversity of spectratyping analysis (donor 1 and 2, 11 mo, blood)

Donor	B cell subset	Transcripts	Peak analyzed[a] (H-CDR3 size[b])	Total number of sequences	Number of different VDJ junctions	Clonal diversity
D1	IgD^+CD27^+	$V_H6\ \mu$	No. 1 (10 aa)	33	30	91%
			No. 2 (11 aa)	24	21	87%
			No. 3 (12 aa)	32	28	87%
			No. 4 (13 aa)	15	13	87%
D1	Naive	$V_H6\ \mu$	No. 1 (12 aa)	13	9	70%
			No. 2 (13 aa)	20	15	75%
			No. 3 (14 aa)	9	9	100%
			No. 4 (15 aa)	14	12	86%
D1	Switched	$V_H6\ \gamma$	No. 1 (11 aa)	19	6	31%
			No. 2 (12 aa)	21	6	28%
			No. 3 (14 aa)	13	5	38%
			No. 4 (17 aa)	14	1	7%
D2	IgD^+CD27^+	$V_H3.15\ \mu$	No. 1 (9 aa)	22	9	41%
			No. 2 (10 aa)	21	14	66%
			No. 3 (11 aa)	18	12	66%
			No. 4 (12 aa)	21	17	81%
D2	Naive	$V_H3.15\ \mu$	No. 1 (11 aa)	23	12	52%
			No. 2 (12 aa)	20	14	70%
			No. 3 (13 aa)	20	13	65%
			No. 4 (14 aa)	21	13	62%
D2	Switched	$V_H3.15\ \gamma$	No. 1 (11 aa)	24	1	4%
			No. 2 (14 aa)	16	1	6%

[a]The peaks that were analyzed are identified by an asterisk in Fig. 1, and they are numbered in order of appearance from left to right.
[b]H-CDR3 size in aa from the residue after the last V_H-encoded Cys to the last residue before the J_H-encoded Trp.

Table S2. Intrapeak clonal diversity of spectratyping analysis (donor 3, 8 mo, spleen)

B cell subset	Transcripts	Peak analyzed[a] (H-CDR3 size[b])	Total number of sequences	Number of different VDJ junctions	Clonal diversity
IgD^+CD27^+	$V_H3.15\ \mu$	No. 1 (12 aa)	21	21	100%
		No. 2 (13 aa)	19	18	95%
		No. 3 (14 aa)	18	17	94%
Naive	$V_H3.15\ \mu$	No. 1 (15 aa)	29	28	97%
		No. 2 (16 aa)	27	21	77%
		No. 3 (17 aa)	15	15	100%
IgD^-CD27^+ (Switched + GC)	$V_H3.15\ \gamma$	No. 1 (14 aa)	29	13	45%
		No. 2 (15 aa)	22	15	68%
		No. 3 (16 aa)	22	7	32%
$CD38^+CD24^-$ (GC)	$V_H3.15\ \mu$	No. 1 (13 aa)	20	10	50%
		No. 2 (14 aa)	19	12	63%
		No. 3 (15 aa)	23	13	57%
		No. 4 (16 aa)	23	13	57%
		No. 5 (17 aa)	22	11	50%
		No. 6 (20 aa)	26	12	46%
$CD38^+CD24^-$ (GC)	$V_H3.15\ \gamma$	No. 1 (12 aa)	16	5	31%
		No. 2 (13 aa)	23	13	57%
		No. 3 (14 aa)	20	13	65%
		No. 4 (15 aa)	19	9	47%
		No. 5 (16 aa)	20	11	55%

[a]The peaks that were analyzed are identified by an asterisk in Fig. 2, and they are numbered in order of appearance, from left to right.

[b]H-CDR3 size in aa, from the residue after the last V_H-encoded Cys to the last residue before the J_H-encoded Trp.

Table S3. Intrapeak clonal diversity of spectratyping analysis (donor 3, 8 mo, spleen)

B cell subset	Transcripts	Peak analyzed[a] (H-CDR3 size[b])	Total number of sequences	Number of different VDJ junctions	Clonal diversity
IgD$^+$CD27$^+$	V_H6 μ	No. 1 (10 aa)	23	20	87%
		No. 2 (11 aa)	16	16	100%
		No. 3 (12 aa)	19	14	73.5%
		No. 4 (14aa)	10	8	80%
Naive	V_H6 μ	No. 1 (12 aa)	21	15	71.5%
		No. 2 (13 aa)	21	13	62%
		No. 3 (15 aa)	14	8	57%
IgD$^-$CD27$^+$ (Switched + GC)	V_H6 γ	No. 1 (11 aa)	16	6	37.5%
		No. 2 (13 aa)	15	4	26.5%
		No. 3 (17 aa)	19	2	10.5%
CD38$^+$CD24$^-$ (GC)	V_H6 μ	No. 1 (10 aa)	24	2	8.5%
		No. 2 (14 aa)	16	3	18.75%
		No. 3 (17 aa)	15	3	20%
CD38$^+$CD24$^-$ (GC)	V_H6 γ	No. 1 (13 aa)	13	1	7.7%
		No. 2 (16 aa)	24	1	4.2%
		No. 3 (17 aa)	20	2	10%
		No. 4 (20 aa)	19	1	10.5%

[a]The peaks that were analyzed are identified by an asterisk in Fig. 2, and they are numbered in order of appearance, from left to right.
[b]H-CDR3 size in amino acids (aa), from the residue after the last V_H-encoded Cys to the last residue before the J_H-encoded Trp.

Table S4. V_H5.51 repertoire analysis of the splenic B cell subsets of a 23-mo-old child (D7)

B cell subset	Transcripts		Peak analyzed[a] (H-CDR3 size[b])	Total number of sequences	Number of different VDJ junctions	Clonal diversity	P value[c]
IgD$^+$CD27$^+$	$V_H5.51$ μ		No. 1 (11 aa)	19	19	100%	
			No. 2 (12 aa)	20	20	100%	
			No. 3 (14 aa)	22	20	91%	
			No. 4 (15 aa)	17	12	70.5%	
		Total:	4	78	71	91%	
Naive	$V_H5.51$ μ		No. 1 (12 aa)	20	19	95%	
			No. 2 (14 aa)	19	14	73.5%	
			No. 3 (16 aa)	19	18	94.5%	
			No. 4 (18 aa)	13	13	100%	
		Total:	4	71	64	90%	
IgD$^-$CD27$^+$ (Switched + GC)	$V_H5.51$ γ		No. 1 (11 aa)	18	9	50%	
			No. 2 (12aa)	22	10	45.5%	
			No. 3 (13aa)	15	8	53.5%	
			No. 4 (14 aa)	15	5	33.5%	
		Total:	4	70	32	45%	3×10^{-9}

[a]The peaks that were analyzed are identified by an asterisk in Fig. S1, and they are numbered in order of appearance from left to right.
[b]H-CDR3 size in aa, from the residue after the last V_H-encoded Cys to the last residue before the J_H-encoded Trp.
[c]Statistical significance (see Materials and methods) of differences in clonal diversity between IgD$^+$CD27$^+$ and IgD$^-$CD27$^+$ cells of the same donor.