Supporting Information

Mobilization of tissue-resident memory CD4+ T lymphocytes and their contribution to a systemic secondary immune reaction

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Supporting Information Tables

Table S1. Key resource table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-CD3 APC/Cy7	Biolegend	Cat#344818; RRID: AB_10645474
anti-CD8 APC/Cy7	Biolegend	Cat#301016; RRID: AB_314134
anti-CD8 BV785	Biolegend	Cat#301046; RRID: AB_2563264
anti-CD45 FITC	Biolegend	Cat#304006; RRID: AB_314394
anti-CD45RA BV570	Biolegend	Cat#304132; RRID: AB_314410
anti-CD56 PE	Biolegend	Cat#318306; RRID: AB_604101
anti-CD69 APC/Cy7	Biolegend	Cat#310914; RRID: AB_314849
anti-CD154 BV421	Biolegend	Cat#310824; RRID: AB_2562721
anti-CCR7 A488	Biolegend	Cat#353206; RRID: AB_10916389
anti-IFNγ PE/Cy7	Biolegend	Cat#502528; RRID: AB_2123323
anti-IL-2 APC/Cy7	Biolegend	Cat#500342; RRID: AB_2562855
anti-IL-2 FITC	Biolegend	Cat# 500304; RRID: AB_315091
anti-CD19 BV421	BD Horizon	Cat# 562440
anti-TNFα APC	BDPharminogen	Cat# 551384
anti-CD4 PE/Cy5.5	eBioscience	Cat# 35-0047-42; RRID: AB_11218283
anti-KI67 PE	eBioscience	Cat#12-5699-42; RRID: AB_10688373
anti-CD3 FITC	In house	Clone UCHT1
anti-CD4 PE	In house	Clone TT1
anti-CD8 PE/Cy7	In house	Clone GN11/134D7
anti-CD14 PO	In house	Clone TM1
anti-CD19 PO	In house	Clone BU12
anti-CD3 PerCP	Miltenyi Biotec	Cat# 130-094-965
anti-CD127 PE	Miltenyi Biotec	Cat# 130-109-435
Chemicals, Peptides, and Recombinant Proteins		
Buffer EL	Qiagen	Cat# 79217
5X Lyse/Fix Solution	BD Biosciences	Cat# 558049

BD FACS Lysing solution	BD Biosciences	Cat# 349202
BD FACS Permeabilizing solution 2	BD Biosciences	Cat# 347692
Brefeldin A	Biolegend	Cat# 420601
(MMR) live attenuated vaccine-Priorix®	GSK	N/A
Qiazol Lysis Reagent	Qiagen	Cat# 79306
X-VIVO 15 Chemically defined medium	Lonza	Cat# BE02-060F
CD28 pure - functional grade	Miltenyi Biotec	Cat# 130-093-375
CD40 pure - functional grade	Miltenyi Biotec	Cat# 130-094-133
FcR blocking reagent	Miltenyi Biotec	Cat# 130-059-901
CMV-pp65	Miltenyi Biotec	Cat# 130-091-824
Measles	Microbix Biosystems	Cat# EL-04-02-001
Mumps	Microbix Biosystems	Cat# El-06-02-001
Rubella	Microbix Biosystems	Cat# EL-05-11-001
Tetanus Toxoid	NIBSC	Cat# 02/232
Q5 High-Fidelity DNA Polymerase	New England Biolabs	Cat# M0491L
Uracil-DNA glycosilase (UDG)	New England Biolabs	Cat# M0280L
Proleukin (IL-2 clinical use)	Novartis	N/A
Human male Ab serum	Sigma-Aldrich	Cat# H4522-100ML
CellTrace™ CFSE Cell Proliferation Kit	ThermoFischer	Cat# C34554
Live/Dead™Fixable Aqua Dead Cell Stain Kit (Pacific Orange)	ThermoFischer	L34957
Penicillin-Streptomycin-Glutamine (100X)	ThermoFischer	10378016
RPMI Medium 1640 - GlutaMax	ThermoFischer	Cat# 21875-091
RNAse inhibitor	ThermoFischer	Cat# N8080119
Critical Commercial Assays		
SMARTer® PCR cDNA synthesis	Clontech	Cat# 634926
TruSeq DNA PCR-Free Library Prep	Clontech	Cat# 20015962
miRNAeasy Micro Kit	Qiagen	Cat# 217084
MinElute PCR Purification Kit	Qiagen	Cat# 28004
QIAquick PCR purification kit	Qiagen	Cat# 28104
PCR-clean-up Gel extraction	MACHEREI-NAGEL	Cat# 740587
Measles virus IgG ELISA	IBL International	Cat# RE57141
Measles virus IgM µ-capture ELISA	IBL International	Cat# RE57151
Mumps virus (Parotitis) IgG ELISA	IBL International	Cat# RE56641
Mumps virus (Parotitis) IgM ELISA	IBL International	Cat# RE56651

Rubella virus IgG ELISA	IBL International	Cat# RE57081
Rubella virus IgM μ-capture ELISA	IBL International	Cat# RE57091
Tetanus virus IgG ELISA	IBL International	Cat# RE56901
CD154 MicroBead Kit	Miltenyi Biotec	Cat# 130-092-658
Inside stain kit	Miltenyi Biotec	Cat# 130-090-477
Oligonucleotides		
1st strand cDNA synthesis		
SmartNNNa: template switch adapter with unique molecular identifier (UMI): AAGCAGUGGTAUCAACGCAGAGU NNNN U NNNN U NNNNN UCTT gggg	Metabion	(Mamedov et al., 2013)(1)
bc1R: primer for cDNA synthesis, human TCR beta mRNA CAG TAT CTG GAG TCA TTG A	Metabion	(Mamedov et al., 2013)
1st PCR amplification		
bc2R: nested primer 1, human TCR beta library TGC TTC TGA TGG CTC AAA CAC	Metabion	(Mamedov et al., 2013)
Step-out primers 1 with sample barcodes. Anneals on the template switch adapter		(Mamedov et al., 2013)
Na-SB2-M1: CGA GCG TGA CGA CGA CAG TAG TCG TGG TAT CAA CGC AGA GT	Metabion	(Mamedov et al., 2013)
Na-SB4-M1: CGA GCG TGA CGA CGA CAG TCA TCG TGG TAT CAA CGC AGA GT	Metabion	(Mamedov et al., 2013)
Na-SB5-M1: CGA GCG TGA CGA CGA CAG GAT TCG TGG TAT CAA CGC AGA GT	Metabion	(Mamedov et al., 2013)
Na-SB6-M1: CGA GCG TGA CGA CGA CAG GTC TTG TGG TAT CAA CGC AGA GT	Metabion	(Mamedov et al., 2013)
Na-SB7-M1: CGA GCG TGA CGA CGA CAG AGT CTG TGG TAT CAA CGC AGA GT	Metabion	(Mamedov et al., 2013)
Na-SB8-M1: CGA GCG TGA CGA CGA CAG ACT TCA GTG GTA TCA ACG CAG AGT	Metabion	(Mamedov et al., 2013)
Na-SB9-M1: CGA GCG TGA CGA CGA CAG ATC CTA GTG GTA TCA ACG CAG AGT	Metabion	(Mamedov et al., 2013)
Na-SB10-M1: CGA GCG TGA CGA CGA CAG CAA CTT GTG GTA TCA ACG CAG AGT	Metabion	(Mamedov et al., 2013)

Na-SB11-M1: CGA GCG TGA CGA CGA CAG CCT AAT GTG GTA TCA ACG CAG AGT	Metabion	(Mamedov et al., 2013)
Na-SB12-M1: CGA GCG TGA CGA CGA CAG CGG TCT GTG GTA TCA ACG CAG AGT	Metabion	(Mamedov et al., 2013)
Na-SB13-M1: CGA GCG TGA CGA CGA CAG CTC GGT GTG GTA TCA ACG CAG AGT	Metabion	(Mamedov et al., 2013)
Na-SB14-M1: CGA GCG TGA CGA CGA CAG GCT CTG GTG GTA TCA ACG CAG AGT	Metabion	(Mamedov et al., 2013)
Na-SB16-M1: CGA GCG TGA CGA CGA CAG TAA TCC GTG GTA TCA ACG CAG AGT	Metabion	(Mamedov et al., 2013)
Na-SB17-M1: CGA GCG TGA CGA CGA CAG TCT GGC GTG GTA TCA ACG CAG AGT	Metabion	(Mamedov et al., 2013)
Na-SB18-M1: CGA GCG TGA CGA CGA CAG TGG CTC GTG GTA TCA ACG CAG AGT	Metabion	(Mamedov et al., 2013)
Na-SB19-M1: CGA GCG TGA CGA CGA CAG TTC AAC GTG GTA TCA ACG CAG AGT	Metabion	(Mamedov et al., 2013)
Na-SB20-M1: CGA GCG TGA CGA CGA CAG GTC TCG GTG GTA TCA ACG CAG AGT	Metabion	(Mamedov et al., 2013)
2nd PCR amplification		
Nested primer 2, human TCR beta. Anneals to the switch adapter		
N2Na: NNC GAG CGT GAC GAC GAC AG	Metabion	(Mamedov et al., 2013)
N3Na: NNN CGA GCG TGA CGA CGA CAG	Metabion	(Mamedov et al., 2013)
N4Na: NNN NCG AGC GTG ACG ACG ACA G	Metabion	(Mamedov et al., 2013)
Reverse primer for TCR beta chain with sample barcodes	Metabion	(Mamedov et al., 2013)
bc3R-SB2r: TAG TCA CAC STT KTT CAG GTC CTC	Metabion	(Mamedov et al., 2013)
bc3R-SB4r: TCA TCA CAC STT KTT CAG GTC CTC	Metabion	(Mamedov et al., 2013)

bc3R-SB5r: GAT TCA CAC STT KTT CAG GTC CTC	Metabion	(Mamedov et al., 2013)
bc3R-SB6r: GTC TTA CAC STT KTT CAG GTC CTC	Metabion	(Mamedov et al., 2013)
bc3R-SB7r: AGT CTA CAC STT KTT CAG GTC CTC	Metabion	(Mamedov et al., 2013)
bc3R-SB8r: ACT TCA ACA CST TKT TCA GGT CCT C	Metabion	(Mamedov et al., 2013)
bc3R-SB9r: ATC CTA ACA CST TKT TCA GGT CCT C	Metabion	(Mamedov et al., 2013)
bc3R-SB10r: CAA CTT ACA CST TKT TCA GGT CCT C	Metabion	(Mamedov et al., 2013)
bc3R-SB11r: CCT AAT ACA CST TKT TCA GGT CCT C	Metabion	(Mamedov et al., 2013)
bc3R-SB12r: CGG TCT ACA CST TKT TCA GGT CCT C	Metabion	(Mamedov et al., 2013)
bc3R-SB13r: CTC GGT ACA CST TKT TCA GGT CCT C	Metabion	(Mamedov et al., 2013)
bc3R-SB14r: GCT CTG ACA CST TKT TCA GGT CCT C	Metabion	(Mamedov et al., 2013)
bc3R-SB16r: TAA TCC ACA CST TKT TCA GGT CCT C	Metabion	(Mamedov et al., 2013)
bc3R-SB17r: TCT GGC ACA CST TKT TCA GGT CCT C	Metabion	(Mamedov et al., 2013)
bc3R-SB18r: TGG CTC ACA CST TKT TCA GGT CCT C	Metabion	(Mamedov et al., 2013)
bc3R-SB19r: TTC AAC ACA CST TKT TCA GGT CCT C	Metabion	(Mamedov et al., 2013)
bc3R-SB20r: GTC TCG ACA CST TKT TCA GGT CCT C	Metabion	(Mamedov et al., 2013)
Software and Algorithms		

BD FACSDiva 6	BD Biosciences	
MACSQuantify^{TM} software	Miltenyi Biotec	
Flowjo 9.9.4 / 10.2	Tree Star	
GraphPad Prism 5.x	GraphPad Software	

References

1.   I. Z. Mamedov *et al.*, Preparing unbiased T cell receptor and antibody cDNA libraries for the deep next generation sequencing profiling. *Frontiers in Immunology* **4** (2013).

Table S2. Study subjects

Donor	Age (years)	Gender	Antibody titers pre-vaccination		
			Measles (mIU/mL) Cut off: 220	Mumps (U/mL) Cut off: 350	Rubella (IU/mL) Cut off: 10)
MMR1	28	F	400	700	161
MMR2	34	F	3300	2200	42
MMR3	32	F	1400	2600	44
MMR4	29	F	-ve	2500	424
MMR5	26	F	1900	860	44
MMr6	43	F	9200	2500	95
MMR7	42	M	11000	4400	26
MMR8	31	M	920	3800	187
MMR9	38	M	360	350	16
MMR10	37	M	4200	4600	107
MMR11	34	F	+ve	+ve	+ve
Ctrl1	57	M	+ve	+ve	237.50
Ctrl2	34	F	+ve	+ve	127.50
Ctrl3	24	F	+ve	+ve	-ve
Ctrl4	31	F	+ve	+ve	440.5

Information about age, gender, and measles, mumps, and rubella virus antigen-specific antibody titers of study subjects pre-vaccination. –ve, negative; +ve, positive.

Table S3. Sequencing metadata for TCR CDR3 Vβ repertoires.

Sample	Replicates (N° cells)		Mapped reads		UMI counts		Clonotypes	
	a	b	a	b	a	b	a	b
MMR10_d0	2500	2500	749116	266776	175	269	106	150
MMR10_d1	2500	2500	651958	242883	638	523	360	282
MMR10_d14	2500	2500	284930	367200	783	763	395	393
MMR11_d0	2500	2500	356605	194558	376	399	302	314
MMR11_d1	2500	2500	587611	263078	773	835	558	602
MMR11_d14	2500	2500	849199	300429	404	463	306	343

PBMCs from vaccinees MMR10 and MMR11 stimulated with measles and two replicates (a and b) and from each 2,500 antigen-reactive $CD154^+CD69^+$ memory $CD4^+$ T cells were isolated before (d0) and 1 (d1) and 14 (d14) days after MMR vaccination. Total RNA was extracted from each set of cells and subjected to parallel library preparation. The mapped reads, the counts of unique molecular identifier (UMI; tagging the quantity of unique mRNA transcripts), CDR3 clonotypes from both replicates are shown.