Extended antigen specific cellular responses by single-cell immune profiling

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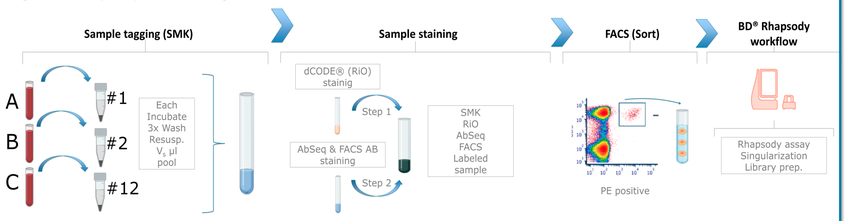
Abstract:

Evaluating the overall antigen-specific cellular immunity of an individual is important for understanding anti-tumor immunity and for development of personalized vaccines and immunotherapy.

The dCODE Dextramer® (RiO) and dCODE Klickmer®, technology together with the BD Rhapsody™ Single-Cell Analysis workflow combine single-cell genomic profiling with antigen-specific recognition allowing deep pheno- and genotypic analysis of antigen-specific T and B cell. However, to unveil the heterogenicity of the cellular immune responses seen in cancer and infectious disease many specificities need to be investigated to get the complete picture.

Here we report how multiple samples can be assayed for antigen specific: T cells, MAIT, iNKT cells, and B cells responses, simultaneously in a single workflow.

Fig1: Sample processing



Experimental setup:

We sample tagged 3 different cell samples individually, and composed a single sample in which we would demonstrate detection of antigen specific cells in the 5 different immune cell types: CD8+ cytotoxic T cells, CD4+ T helper cells, iNKT cells, MAIT cells, and B cells (Fig.12).

The 3 samples and sample tag (SMK):

SMK1: A healthy pre-COVID-19 HPBMC sample with several known virus specific cytotoxic CD8 T cells.

SMK2: A CD4 enriched cell sample, stimulated and expanded in the presence of a EBV specific peptide.

SMK12: A normal HPBMC sample form a previously COVED-19 infected and vaccinated healthy Donor.

The cells were mixed in a ratio of app. 10(SM1) to 1(SMK2) to 15(SMK12) respectively, then stained with a panel of dCODE Dextramer ® (RiO) reagents (Table 1), and thereafter the Immune Discovery Panel (IDP), containing 30 BD® AbSeq assay (Table 2).

dCODE Dextramer® positive cells (PE+) were sorted, and the cells were subjected to the BD Rhapsody™ System for full length TCR/BCR, VDJ sequencing, BD® AbSeq assay and dCODE Dextramer® (RiO) Library Preparation, and DNA sequencing. After sequencing of the libraries, the seq. data were processed through the Seven Bridges, BD Rhapsody™ Sequence Analysis Pipeline and subsequent analyzed using BD SeqGeq™ software package.

Data analysis

The data from the composed sample were analyzed in whole for its canonical markers and gated for detection of each of the antigen specific cell types (Fig.2 a-e). A "canonical marker" parameter were generated containing CD3, CD4, CD8, CD19, and CD161, plotted against CD14[a], to remove monocytes from the downstream analysis. The CD14- cells were gated for CD3+ and CD19+ to separate the T cells from the B cells[b], and the CD4+ and CD8+ positive T cells were subsequent gated from the CD3 positive population[c]. The CD14- cells were furthermore gated for CD161+ and MR1(5-OP-RU)+ antigen specific MAIT cells[d], and for CD1d(α-GalSer)+ iNKT[e] cells.

Each of the CD4+, CD8+ and CD19+ populations were analyzed for the presence of antigen specific cells defined in the Dextramer® panel (Tabel1, Fig.2, f-g plots). The antigen specific cells were immunophenotyped using the IDP panel (Tabel 2) and defined as being, Naïve, Naïve like, Effector cells, Central memory cells and Effector memory cells (Fig.4). The found antigen specific cells were furthermore defined by its full length clonotype (Fig.6).

Fig 2: Gating main cell types, and identification of antigen specific cells

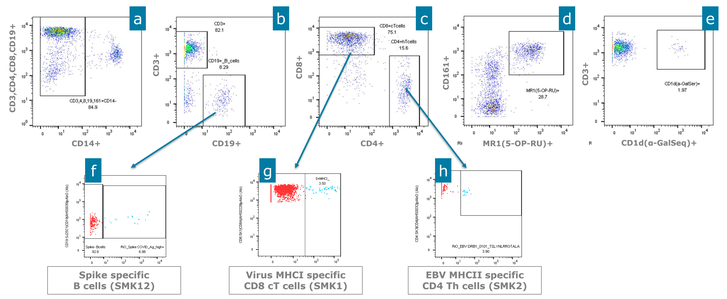
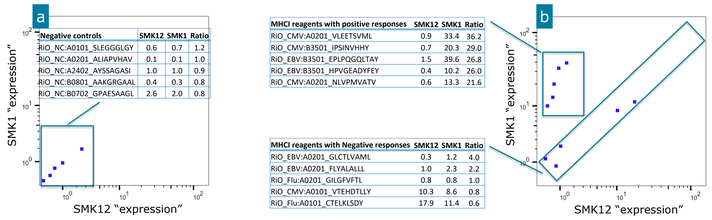


Fig 2: a-e gating of the 5 immune cell types in the composed sample; a) Exclusion of CD14+ monocytes, b) CD3+ T cells & CD19+ B cells, C) CD4+ and CD8+ T cells, d)CD161+MR1+MAIT cells, and e) CD3+CD1d+iNKT cells.
Lower f-h): Identification of the antigen specific T and B cells, overlayed (Blue) of the deconvoluted sample tag (single sample) antigen specific cells. Identifying the same cells in the composed sample, as in the sample tag deconvoluted samples (blue).

Fig 3: Use of internal neg. cell control to define antigen specific CD8 T cells



Negative controls	SMK12	SMK1	Ratio
RiO_NC:A0101_SLEGGGLGY	0.6	0.7	1.2
RiO_NC:A0201_ALIAPVHAV	0.1	0.1	1.0
RiO_NC:A2402_AYSSAGASI	1.0	1.0	0.9
RiO_NC:B0801_AAKGRGAAL	0.4	0.3	0.8
RiO_NC:B0702_GPAESAAGL	2.6	2.0	0.8

MHCI reagents with positive responses	SMK12	SMK1	Ratio
RiO_CMV:A0201_VLEETSVML	0.9	33.4	36.2
RiO_CMV:B3501_IPSINVHHY	0.7	20.3	29.0
RiO_EBV:B3501_EPLPQGQLTAY	1.5	39.6	26.8
RiO_EBV:B3501_HPVGEADYFEY	0.4	10.2	26.0
RiO_CMV:A0201_NLVPMVATV	0.6	13.3	21.6

MHCI reagents with Negative responses	SMK12	SMK1	Ratio
RiO_EBV:A0201_GLCTLVAML	0.3	1.2	4.0
RiO_EBV:A0201_FLYALALLL	1.0	2.3	2.2
RiO_Flu:A0201_GILGFVFTL	0.8	0.8	1.0
RiO_CMV:A0101_VTEHDTLLY	10.3	8.6	0.8
RiO_Flu:A0101_CTELKLSDY	17.9	11.4	0.6

Fig.3: Sample tag (SMK) can be used to introduce an internal cell control for defining antigen specific responses. In this experiment we wanted to identify MHCI responses in an allele matched sample tagged donor (SMK1). We used a donor with no overlapping alleles, sample tagged with SMK12 as internal cellular control. CD8+ T cells were identified in each donor as a population, and the "gene expression / parameter density" of each was plotted in gene-view, CD8+SMK1 vs CD8+SMK12 expression, and then parameters were selected for either:
The 5 negative control dCODE MHCI Dextramer ® reagents or b) The analytic dCODE MHCI Dextramer® virus specificities.
a) No differences are observed between CD8+ population of SMK1 and SMK12 for the negative control reagents.
b) 5 easily identifiable MHCI responses are "overexpressed" in SMK1 vs SMK12. Five MHCI specificities were found negative in SMK1 vs SMK12 plot. The same responses were found / not found when evaluating each MHCI specificity against CD8+ individually (data not shown).

Fig 4: Phenotyping antigen specific B-cells, CD8+ T cells and CD4+ T cells

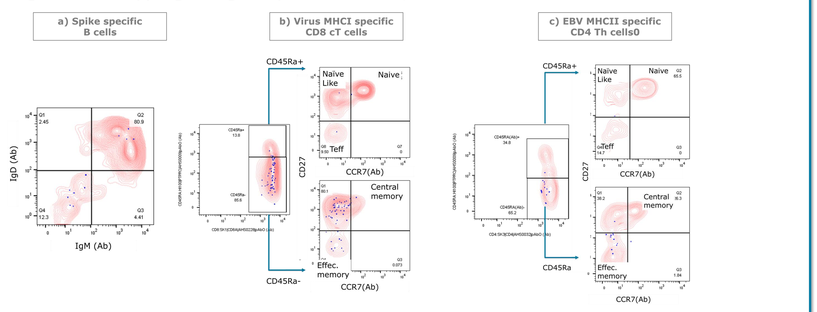
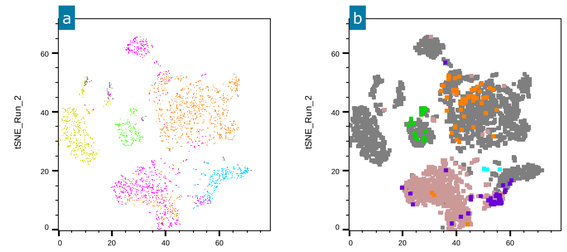


Fig 4: Phenotyping antigen specific cells; B-Cells, CD8 T Cells, and CD4 T cells using BD® AbSeq assay antibodies. The antigen specific cells overlayed on the antigen negative cells, are shown.
a) Most of the identified COVID-19, Spike Specific B cells are IgM and IgD negative, indication B cells type shifting toward mature blast cells. b) The Virus specific CD8 T cells differ from the main T cell population by being primarily CD45 and CCR7 negative, and CD27+, being Central memory cells. c) The EBV CD4 T cells (very few) are CD45, and CD27- having an effector Memory phenotype.

Fig 5: Phenotyping and placing the antigen specific cells on the cellular atlas



Subset Name	Count
CD56+ NK-cells	746
CD14+ monocyts	452
CD19+ _B_cells	215
CD8+cTcells	1599
CD4+hTcells	333
Clean_cells	3054

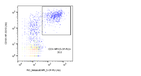
Subset Name	Count
5+MHCI_	56.0
RiO_Spike:COVID_Ag_high+	15.0
RiO_EBV:DRB1_0101_TSLYNLRRGTALA	13.0
CD1d(a-GalSer)+	42.0
MR1(5-OP-RU)+	743
Clean_cells	3054

Fig 5: PCA was performed on all parameters, and the Principal components with a variance >1% were used to generate the t-SNE plot.
a) t-SNE with overlay of the major cell types defined by BD® AbSeq assay antibodies.
b) t-SNE with overlayed antigen specific responses, showing:
The dCODE MHCI Dextramer®+ cells are colocalized within the CD8+CD56- population, the COVID-19 SPIKE+ B-Cells within the CD19+B-Cell pop. The EBV class II positive cells are within the CD4+ populations. CD1d(α-GalSeq)+ iNKT cells are mainly in the CD8+CD56+ pulping of NK-cells, and some within the CD4+ T cell population. MR1(5-OP-RU)+ cells, are all within the CD8+ CD56+ T cell population and seems to be the driver in segregating the CD8+ cells into two populations of the t-SNE plots.

Fig 6: Results of Antigen-specific Cell Clonal analysis:

Populations of antigen-specific CD4+ helper, and CD8+ cytotoxic T cells, Spike specific B cells, as well as iNKT and MAIT cells, were identified, and the full length VDJ sequences were analyzed. VDJ composition of the paired T cell and B cell receptors are shown.

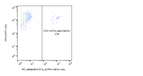
CD161+ MR1(5-OP-RU) MAIT cells



MAIT cells	Domain seq.	Cell #	%
Epitope	5-OP-RU		
Seq and paired TCR		171	24%
TRAV1-2*01		117	68%
TRAJ33*01		100	85%
α CDR3 most freq. seq.	AVMDSNYQLI	33	33%
Consensus	AxxDSNYQLI	96	
TRBV	TRBV6-4*01	29	30%
	TRBV20-1*01	19	20%
	TRBV6-1*01	13	14%
	TRBV6-2*01	12	13%
TRBD	TRBD1*01	30	31%
	TRBD2*01	28	29%
	TRBD2*02	15	16%
TRBJ	TRBJ2-3*01	26	27%
	TRBJ2-1*01	22	23%
	TRBJ2-7*01	10	10%
	other TRBJ	9	40%

171 cells were sequenced with paired TCR.
Consistence with MAIT cells being "Invariable", we found low variability of the TCR's identified and the major part being TRAV V1-2*01, with low variability of the other domains, as been reported elsewhere[1].
[1]MR1-Restricted T Cells Are Unprecedented Cancer Fighters
Alessandro Vacchini, Andrew Chancellor, Julian Spagnuolo, Lucia Mori and Gennaro De Libero*
Experimental Immunology, Department of Biomedicine, University of Basel and University Hospital Basel, Basel, Switzerland

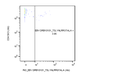
CD3+ CD1d(αGalCer) iNKT cells



iNKT cells	Domain seq.	Cell #	%
Epitope	αGalCer		
Seq and paired TCR		6	14%
	TRAV10*01	5	83%
	TRAJ18*01	5	83%
α CDR3 most freq. seq.	VVSDRGSTLGRLY	5	100%
Consensus	NA		
TRBV	TRBV25-1*01	5	100%
TRBD	NA		
TRBJ	TRBJ1-1*01	1	20%
	TRBJ1-2*01	1	20%
	TRBJ1-5*01	1	20%
	TRBJ2-3*01	1	20%
	TRBJ2-7*01	1	20%

6 cells were sequenced with paired TCR.
Consistence with iNKT cells being "Invariable" T cells, we found low variability of the TCR's identified.
The VDJ alfa region represent only two one V and one J domain and a single α CDR3 seq. the β-VDJ region, represent only one V domain, but 5 different J domains, with variating CDR3 sequences`. Although low number of cells, it show the invariability of at least the α region, and the VDJ usage of both α and β regions.

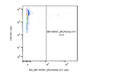
CD4+ MHCII/EBV specific T cells



EBV specific CD4 T cells	Domain seq.	Cell #	%
Epitope	TSLYNLRRGTALA		
Seq and paired TCR		9	69%
TCR α domain	TRAV38-2/DV8*01	9	100%
α CDR3 seq.	AVCNTNAGKST	9	100%
β CDR3 seq.	SVESAGAGDTQY	9	100%
TRBV	TRBV29-1*01	9	100%
TRBD	TRBD1*01	9	100%
TRBJ	TRBJ2-3*01	9	100%

9 cells of a single T cell clone were detected and sequenced with paired TCR.
Consistent with EBV being a endogenous virus, that over time will converge to few antigen specific T cell clones.

CD8+ MHCI/EBV specific T cells

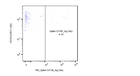


MHCI T cells Virus specific	Cell #	%
Specificity found	35	
HLA-A02/GLCTLVAML	1	3%
HLA-A0201/NLVPMVATV	1	3%
HLA-A0201/ VLEETSVML	9	26%
HLA-B3501/EPLPQGQLTAY	12	34%
HLA-B3501/HPVGEADYFEY	6	17%
HLA-B3501/IPSINVHHY	6	17%

EBV specific CD8 T cells			
Epitope	HLA-B3501/EPLPQGQLTAY		
only one α / β paired domain			CDR3 transl.
α seq.	TRAV12-1*01	(D) NA	TRAJ40*01 VVNMIGSGTYKYI
	TRBV10-3*01	(D) NA	TRBJ2-1*01 AISVSQFDEQF
β seq.			
3	TRBV10-3*01/TRBV19*01	NA	TRBJ1-5*01 AI/SSTGDSNQPQH
3	TRBV10-3*01	NA	TRBJ2-1*01 AISVSQFDEQF
1	TRBV10-3*02	NA	TRBJ2-1*01 ASSDSTNTGELF

The MHC Dextramer® panel generated 37 antigen specific CD8+ T cells. The sequencing or these cells was not optimal, but we could recognize the CDR3 clonotypes of the highest frequent T cell clone

CD19+ B cell Covid-19 Spike



Antigen specific B cells	Domain seq.	Cell #	% paired		
Epitope	COVID-19 Spike	15	67%		
α region					**Transl. seq**
	IGHV3-53*04	IGHD1-26*01	IGHJ3*02	IGHA1	ARDLGNEAFDI
	IGHV1-24*01	IGHD3-22*01	IGHJ4*02	IGHG2	TTYYYDTSGLRYIFDY
	IGHV1-2*02	IGHD3-9*01	IGHJ6*02	IGHG1	AREDILTGAALPPYGLDV
	IGHV3-11*06	IGHD5-12*01	IGHJ4*02	IGHG1	ARGDLPTFGNSGYDWGRTFDY
	IGHV3-30-3*01	IGHD3/OR15-3a*01	IGHJ5*02	IGHG1	ARGWTALGVFSWFDP
β region					
	IGKV1-9*01	IGKJ2*04	IGKC	QQLNSYPPGS	
	IGLV7-46*01	IGLJ2*01	IGLC2	LLSYSGAREV	
	IGKV2-24*01	IGKJ1*01	IGKC	MQATQLLTWT	
	IGLV1-51*01	IGLJ2*01	IGLC2	GTWDSSLNVV	

We identified 15 COVID Spike specific B cells in this sample. The variable domains are significant different between the B-cell clones.

Summary:

We here demonstrate analysis of antigen-specific immune responses in 5 antigen-specific immune cell types: CD8+ cytotoxic T cells, CD4+ T helper cells, iNKT, and MAIT cells, as well as B cells, using a single workflow on a composed sample, in which each cellular component is sample tagged, individually.

Multiple virus specific responses were identified at the single cell level and cells characterized based on receptor recognition and full length VDJ clonotype sequence. Full length VDJ TCR/BCR parried clonotypes were identified for each of the responses identified.

The antigen responses are found with equal resolution and sensitivity, whether on the background of the whole composed sample or the SMK deconvoluted individual samples, indicating that mixing multiple sample do not decreasing the overall sensitivity of the assay.

Using sample tagging to introduce an internal negative control sample in the assay, enables identification of antigen specific T cells with high specificity, based on a "differential expression" analysis between the two sample tagged samples, showing that sample tagging can be an efficient tool where an internal cellular control can be introduced in the assay.

Furthermore, we enable phenotypic characterization of the antigen specific cells, using a 30-member immune response panel of BD®AbSeq antibodies (IDP), as well as placed the found antigen specific cells within each of the overall immune cell types, showing antigen specific cells were identified only in the cellular compartment that makes biological sense.

This work was sponsored by BDbiosciences®

Tabel 1: dCODE Dextramer® (RiO) reagent panel.

Cat.No.	dCODE Dextramer® (RiO) pool:	Antigen	Cell type
FA10002	HLA-DRB1*0101/PVSKMRMATPLLMQA (CLIP)	NC	CD4 T helper cell
FA10003	DRB1*0101/TSLYNLRRGTALA (EBV)	EBV	CD4 T helper cell
WA3580	HLA-A0101/SLEGGGLGY	NC	Cytotoxic T cell
WB2666	HLA-A0201/ALIAPVHAV	NC	Cytotoxic T cell
WF3231	HLA-A2402/AYSSAGASI	NC	Cytotoxic T cell
WH3397	HLA-B0702/GPAESAAGL	NC	Cytotoxic T cell
WI3233	HLA-B0801/AAKGRGAAL	NC	Cytotoxic T cell
WB2161	HLA-A0201/GILGFVFTL	Flu	Cytotoxic T cell
WB2130	HLA-A02/GLCTLVAML	EBV	Cytotoxic T cell
WB3529	HLA-A02/FLYALALLL	EBV	Cytotoxic T cell
WB2132	HLA-A0201/NLVPMVATV	CMV	Cytotoxic T cell
WB2658	HLA-A0201/ VLEETSVML	CMV	Cytotoxic T cell
WK2146	HLA-B3501/EPLPQGQLTAY	EBV	Cytotoxic T cell
WK2145	HLA-B3501/HPVGEADYFEY	EBV	Cytotoxic T cell
WK2138	HLA-B3501/IPSINVHHY	CMV	Cytotoxic T cell
WA2131	HLA-A0101/VTEHDTLLY	CMV	Cytotoxic T cell
WA3410	HLA-A0101/CTELKLSDY	Flu	Cytotoxic T cell
	dCODE Klickmer® (RiO)/COVID-19 Spike protein	B Cell Ag	B Cell
	dCODE Klickmer® (RiO)/MR1(5-OP-RU)	Metabolit	MAIT cell
XD8002	hCD1d (α-GalCer)	Metabolit	iNKT cell
XD8001	hCD1d (unloaded)	NC	iNKT cell

*NC: Negative control

Tabel 2: BD® AbSeq Immune Discovery Panel

Specificity	Clone	Oligo ID Cell type / phenotype
CD3	UCHT1	AHS0231 canonical T Cell marker
CD4	SK3	AHS0032 canonical T helper Cells
CD8	SK1	AHS0228 canonical Cytotoxic T Cells
CD11c	B-Ly6	AHS0056 Dendritic cell marker
CD14	M9HIP9	AHS0037 canonical Monocytic marker
CD16	3G8	AHS0053 Natural killer cell marker
CD19	SJ25C1	AHS0030 canonical B cell marker
CD25	2A3	AHS0026 T reg, and T cell activation marker
CD27	M-T271	AHS0025 Differentiation marker
CD28	L293	AHS0138 T cell activation marker
CD45RA	HI100	AHS0009 Naïve cell marker
CD56	NCAM16	AHS0019 Canonic Natural Killer cells
CD62L	DREG-56	AHS0049 Differentiation marker (naive/Central memory cells)
CD127	HIL-7R-M21	AHS0028 Differentiation marker (Effector and memory cells)
CD134	ACT35	AHS0013 T cell Activation marker
CD137	4B4-1	AHS0003 T cell Activation marker
CD161	HP-3G10	AHS0205 MAIT cell marker & Natural killer cell marker
CD183 (CXCR3)	1C6/CXCR3	AHS0031 T cell Activation marker
CD185 (CXCR5)	RF8B2	AHS0039 T Follicular helper (Tfh) cells
CD186 (CXCR6)	13B 1E5	AHS0148 HIV coreceptor
CD196 (CCR6)	11A9	AHS0034 B cell activation marker
CD197 (CCR7)	2-L1-A	AHS0273 Naïve differentiation marker
CD272	J168-540	AHS0052 Naïve T cell marker
CD278	DX29	AHS0012 T cell activation marker
CD279 (PD-1)	EH12.1	AHS0014 T cell exhaustion marker
CD357 (GITR)	V27-580	AHS0104 T cell activation marker
CD366 (TIM-3)	7D3	AHS0016 T cell exhaustion marker
HLA-DR	G46-6	AHS0035 T cells Differentiation / activation marker
IgD	IA6-2	AHS0058 B cell Differentiation marker T cells
IgM	G20-127	AHS0198 B cell Differentiation marker T cells