HLA-Typed PBMC Samples with Established Antigen/Peptide Reactivity for Accelerating and Standardizing Human Immunological Research

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Introduction

We have developed a protocol to cryopreserve human peripheral blood mononuclear cells (PBMC) while maintaining full functionality. The thawed PBMC displayed > 90% viability, and when tested for peptide or protein antigen-induced T cell recall responses in cytokine ELISPOT assays, the frequencies and per-cell cytokine productivities of the thawed cells approximated 100% of the fresh PBMC. Since serum is a highly variable reagent that affects the results, we also developed serum free freezing and testing media (CTL-Cryo™, CTL-Test™) towards standardization. Based on these developments, we are building an immune-characterized PBMC library of HLA-typed healthy human donors intended as positive and negative controls for T cell monitoring in ELISPOT, ELISA, cytokine bead array, tetramer/pentamer, and cytokine capture assays. Ready access to such PBMC should facilitate human immunological research and assay standardization within laboratories, and between different laboratories.

Results

		Fresh PBMC		Frozen PBMC		
Donor	Test antigen	Mean	SD	Mean	SD	P value
A	Media	1.00	0.00	0.30	0.50	0.07
	Mumps	66.00	14.00	66.70	14.99	0.96
	EBV BMLF1 259	73.30	9.07	62.70	11.59	0.28
	Flu Mat1 58	14.00	7.21	18.30	3.51	0.41
B	Media	0.30	0.50	0.00	0.00	0.36
	Flu Mat1 58	19.70	10.02	16.50	9.40	0.71
	EBV EBNA3c 281	77.70	12.58	62.70	12.01	0.21
	HCMV pp65	7.00	1.00	6.50	2.52	0.77
C	Media	0.90	0.78	0.70	0.58	0.74
	Mumps	127.00	11.15	127.30	9.00	0.97
	EBV BMLF1 259	20.30	3.51	15.00	2.16	0.09
	Flu NP 91	18.70	3.51	18.50	4.80	0.96

Table1. *No statistically significant difference between the IFN-γ recall response of fresh and cryopreserved PBMC.* Freshly isolated PBMC were tested in 24h IFN-γ ELISPOT assays at 200,000 cells per well, directly ex vivo ("Fresh PBMC"). The test antigens were 23 individual peptides from a library of 23 MHC-Class I restricted CEF peptides, in addition to the protein antigens Candida, Tetanus, Mumps, Dust Mite, and PPD. After the fresh PBMC had been plated for these assays, the remaining PBMC were cryopreserved in CTL-Cryo™ serum free freezing medium according to CTL protocols ("Frozen PBMC"). These cells were thawed, and were retested under identical conditions as the fresh PBMC, using the serum free testing medium CTL-Test™. Representative data are shown as the mean number of IFN- γ spots induced by the specified antigens for the fresh and the frozen PBMC of 3 donors. The P values on the right show that there is no statistical significant difference between the performance of fresh and frozen PBMC.

Panel A

Subject ID	% Viable	% Recovery
1	95.3	80.8
2	91.4	80.6
3	91.3	87.1
4	91.5	92.7
5	95.3	98.3
6	95.0	82.6
7	93.2	94.8
8	91.3	80.5
9	95.0	99.6
10	93.3	94.7
11	94.6	96.2
12	87.0	97.2
13	89.8	99.4
14	86.7	80.1
15	92.1	84.9
16	92.6	92.7
17	95.4	93.9
18	95.6	99.5

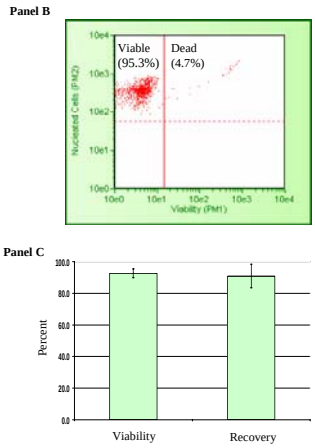

Fig 2. *Viability and recovery rate of cryopreserved PBMC.* Cryopreserved PBMC of 18 donors were thawed according to CTL protocols. (A) The viability of the cells (number of viable cells/total number of cells x 100) was established by viable-dead cell staining using the Guava platform. Also shown are the recovery rates (cell number after thawing /number of cells frozen x 100). (B) A representative plot is shown. (C) The mean and standard deviation of viability and recovery rates for all 18 donors are shown.

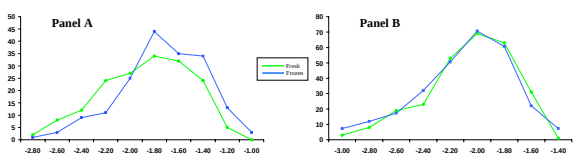

Fig 3. *Cryopreservation does not impair the cytokine productivity of individual CD4 or CD8 T cells.* In cytokine ELISPOT assays, the spot size reflects the cytokine productivity of individual cells. We used an ImmunoSpot® Series 3B Analyzer for the morphometric analysis of IFN-γ spots induced by antigens that trigger CD4 (Panel A) cells or CD8 (Panel B) cells. The spot size distribution for each category is shown for fresh (green) and frozen (blue) cells.

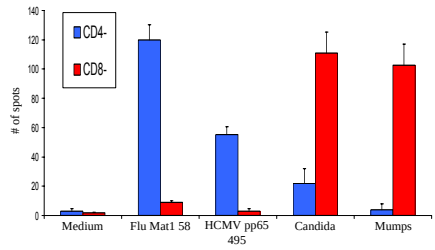

Fig 4. *Defining recall antigen-induced responses as CD4 or CD8 cell mediated.* PBMC were depleted of CD4 (blue) or CD8 (red) cells using magnetic beads, and the cell populations obtained for a donor were tested in an IFN- γ ELISPOT assay for the recall response to the antigens specified. The results show that CD8 cell depletion entirely abrogates the recall response to Flu Mat1 58 and the HCMV pp65 495 peptide but CD4 cell depletion does not affect the recall response identifying the cytokine-producing cells as CD8 cells. Candida and Mumps elicited recall responses by CD4 cells.

Antigen	HLA restriction	Donor 1	Donor 2	Donor 3
		A2, A3 B7,B44, Bw4, Bw6, C7	A2, B18, B51, Bw4, Bw6, C2	A2 A11 B7,B35, Bw6, C4, C7
Media		1 ± 0	0.3 ± 0.58	0 ± 0
CEF pool		251 ± 9.17	352.7 ± 13.32	248.6 ± 58.9
Flu PB 591	A1	1 ± 1	0.3 ± 0.58	0.3 ± 0.58
Flu NP 44	A1	1.3 ± 0.58	0.7 ± 0.58	0.7 ± 0.58
EBV BMLF1 259	A2	62.7 ± 9.07	6 ± 1.53	9.5 ± 3.51
Flu Mat1 58	A2	21.7 ± 7.21	53 ± 11.37	12.8 ± 5.12
HCMV pp65 495	A2	0 ± 0	42 ± 2.94	0.7 ± 1.15
Flu NP 265	A3	9.7 ± 2.08	26 ± 4.58	0.7 ± 1.15
EBV BRLF1 148	A3	4.3 ± 2.08	3.7 ± 3.79	1.3 ± 0.58
EBV EBNA3a 603	A3	2.3 ± 1.15	8.7 ± 3.06	0 ± 0
EBV EBNA3b 416	A11	7.7 ± 4.16	0.3 ± 0.58	3 ± 1
EBV BRLF1 134	A11	0.3 ± 0.58	0.3 ± 0.58	0.7 ± 0.58
EBV BRLF1 28	A24	1.7 ± 1.15	0.3 ± 0.58	0.3 ± 0.58
Flu NP 91	AA68	36.7 ± 5.51	2 ± 1	12.3 ± 3.51
HCMV pp65 417	B0702	3 ± 1	3.3 ± 0.58	2.3 ± 3.21
EBV EBNA3a 379	B7	48.3 ± 14.64	8.3 ± 2.52	18 ± 3.21
EBV EBNA3a 158	B8	2.3 ± 2.31	0.3 ± 0.58	1.3 ± 1.53
EBV EBNA3a 325	B8	9 ± 13.86	1.3 ± 1.53	0.7 ± 0.58
EBV BZLF1 190	B8	0.7 ± 1.15	2 ± 1	0.7 ± 0.58
FLU NP 380	B8	1 ± 1	1 ± 1	0.3 ± 0.58
EBV EBNA3c 258	B27	0.7 ± 0.58	0.7 ± 1.15	0 ± 0
FLU NP 383	B27	1 ± 1	19.7 ± 4.62	0.7 ± 1.15
EBV EBNA3a 458	B35	2 ± 1	0 ± 0	0 ± 0
EBV EBNA3c 281	B44	62.7 ± 12.58	0.3 ± 0.58	2.7 ± 1.53
HCMV pp65	B44	1 ± 0	7 ± 1	0.7 ± 0.58
Candida		280 ± 7.55	78 ± 11.93	18.3 ± 2.52
Mumps		66 ± 14	77.3 ± 26.1	104.7 ± 41.15
Dust Mite Mix		33.3 ± 19.66	6.3 ± 9.29	1.3 ± 0.58
PPD		46 ± 11.53	7.7 ± 4.73	134.8 ± 12.12
Tetanus		30 ± 13.11	3 ± 1.73	11.3 ± 2.31

Fig 5. *Immune characterized cryopreserved PBMC library.* The PBMC of each donor have been characterized for reactivity to a panel of 23 individual peptides (common viral Class I-restricted determinants) and 5 protein recall antigens, recognized by CD8 and CD4 cells, respectively. Up to 1,500 vials of each of the characterized samples have been cryopreserved. To date, samples from 21 donors have been frozen and characterized, continuing at the rate of 2 new donors per week.

Implications:

- Ready access to PBMC: no need for IRB.
- Experiments can be designed *online* based on established HLA-types and antigen reactivity.
- Sizable donor pool facilitates screening for new reactivities and markers.
- Unlimited cell numbers available for experimentation.
- Continuous access to PBMC of the same donor and of the same bleed permit rapid progress with controlled experimentation.
- Experiments can be readily repeated and extended, e.g. if reviewers ask for additional experiments.
- Ability to share the same PBMC with colleagues anywhere helps collaborative work.
- Assay comparisons and validations are facilitated by access to pre-characterized reference samples.
- Availability of positive and negative control samples, e.g., for clinical trials.