How frequently are predicted peptides actually recognized by CD8 cells?

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INTRODUCTION: Detection of antigen-specific CD8 cells relies on the use of peptides that can bind to HLA-Class I molecules. There is extensive knowledge on individual HLA-alleles' peptide binding requirements and for many antigens immunogenic peptides have been defined. The 32 individual peptides that comprise the CEF peptide pool represent such well-defined peptide determinants for Cytomegalo, Epstein Barr, and Flu virus. We tested 42 healthy human donors on the accuracy of these peptide predictions.

For example, will all HLA-A*0201 positive donors who have been infected with one of these viruses show a CD8 cell response to the pre-defined HLA-A*0201-restricted peptide of that virus? If the donor responds, will it be a dominant response, one of several (co-dominant) responses, a weak (subdominant) response, a barely detectable (cryptic) response, or will the peptide not be recognized while responses to other peptides of the virus prevail? How many times are unpredicted peptides of the virus recognized in a dominant fashion? To that practical end, we asked whether reliance on select "immunodominant" peptides is a reliable alternative to agnostic immune monitoring with peptide pools.

METHODS: Forty-two HLA-class I, high-resolution-typed, healthy human donors were selected from the CTL ePBMC® library. The PBMC were tested for reactivity to the individual CEF peptides measuring IFN-γ with the ELISPOT assay. To assure low background, serum-free, CTL-Test™ Medium was used. The spots were counted using an ImmunoSpot® S5 Core reader. The predicted vs. the actually detected response was compared.

RESULTS: Of the expected 241 recall responses, the 32 individual CEF peptides induced a total of 122 positive responses in the 42 donors. Within these 122 positive responses, 36 (30%) were dominant, 41 (34%) were subdominant, and 45 (37%) cryptic. In 119 instances, the predicted peptide was not targeted by CD8 cells detectably. Twenty unpredicted peptides were immune dominant (35%), in 20 instances (35%) unpredicted peptides were subdominant, and in 17 (30%) such peptides elicited weaker, cryptic responses.

CONCLUSIONS: The data clearly shows that predicted peptides are not necessarily immune dominant. In 49% of the test cases, the predicted peptide did not induce a detectable recall response. When it did, it was one of several targeted determinants among which it was subdominant or cryptic. Thus, reliance on one or a few peptides is likely to miss the majority of the antigen-specific CD8 cells, strongly arguing for the use of peptide pools for immune monitoring.

DONOR	HLA DONOR	PREDICTED				UNPREDICTED		
		DOM	SUB	CRYP	NO RSPNS	DOM	SUB	CRYP
1	A68, A68; B8, B35	1	1	5	1	2	0	0
2	A2, A33; B14, B27	2	0	4	2	0	0	0
3	A2, A26; B35, B38	0	2	3	3	0	0	0
4	A2, A2; B60, B49	3	1	1	0	1	0	2
5	A2, A29; B8, B60	1	3	2	3	0	0	0
6	A2, A24; B7, B13	0	2	1	4	0	0	0
7	A2, A29; B35, B44	0	0	0	9	0	0	0
8	A2, A2; B18, B45	0	0	0	6	0	0	0
9	A2, A3; B44, B44	0	0	1	10	0	0	0
10	A2, A2; B60, B44	2	1	1	3	0	2	7
11	A2, A2; B44, B49	0	2	2	2	0	0	0
12	A2, A3; B18, B18	2	1	1	6	0	0	0
13	A2, A30; B39, B44	0	0	0	0	0	0	0
14	A2, A25; B35, B44	2	0	0	7	1	0	1
15	A2, A2; B70, B18	1	1	1	1	0	1	0
17	A2, A32; B62, B44	2	1	2	2	3	2	0
18	A2, A24; B8, B50	0	0	0	0	0	0	0
19	A2, A26; B61, B44	2	1	1	4	4	2	5
20	A1, A68; B57, B57	1	0	1	3	0	0	0
21	A2, A11; B7, B35	1	1	1	3	0	2	0
22	A1, A2; B8, B61	0	4	1	6	1	3	0
23	A2, A24; B61, B44	2	2	1	2	3	4	2
24	A1, A2; B14, B41	0	0	1	4	1	0	0
25	A2, A2; B62, B35	1	0	1	1	0	0	0
26	A2, A3; B7, B8	1	2	2	5	1	0	0
27	A2, A2; B8, B61	0	1	1	1	0	0	0
28	A2, A3; B60, B44	2	2	3	4	1	0	0
29	A2, A11; B60, B44	2	2	1	5	0	0	1
30	A2, A34; B38, B61	1	0	0	0	0	0	1
31	A3, A3; B14, B62	0	0	0	0	0	0	0
32	A24, A32; B8, B55	1	1	2	1	1	0	0
33	A2,A3; B7, B27	2	3	1	7	0	0	0
34	A2, A32; B38, B60	0	0	0	0	0	0	0
35	A2, A3; B27, B62	0	0	0	0	0	0	0
36	A2, A68; B7, B44	0	0	0	0	0	0	0
37	A2, A24; B39, B46	0	0	0	1	0	0	0
38	A2, A33; B27, B65	1	0	0	0	0	0	0
39	A2, A2; B8, B61	0	2	1	5	0	0	0
40	A4, A12; B15, B51	0	0	0	0	0	0	0
41	A2, A3; B7, B44	1	2	1	6	0	0	0
42	A2, A2; B44, B48	1	3	0	1	1	4	0
43	A2, A33; B35, B44	1	0	0	1	0	0	0

Table 2: **Detected vs. predicted responses to CEF peptides for 42 donors.** Using an IFN-γ ELISPOT assay, the PBMC were tested for reactivity to the individual 32 CEF peptides. A predicted response is when one of the HLA alleles expressed by a donor matches the pre-defined HLA restriction of a CEF peptide (see Table 1). An unpredicted response is defined as a CEF peptide inducing a recall response while the HLA molecule that was originally described as the restriction element of this peptide is not expressed by the donor. The responses were defined as immune dominant (>100 spots/400,000 PBMC), subdominant (30-100 spots), or cryptic (10-29 spots/400,000 PBMC). The number of predicted and unpredicted responses falling in the dominant (DOM), subdominant (SUB) and cryptic (CRYP) category are shown for each donor.

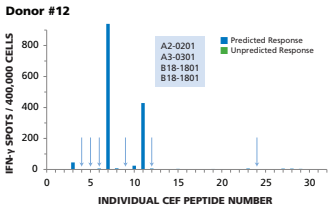

Figure 1: **Example of immune dominance for two predicted peptides and lack of response to six other predicted peptides.** Based on the HLA-type of this donor (shown in the insert), one would expect responses to ten CEF peptides: CEF-3 and CEF-4 (A2 restricted flu peptides), CEF-5 and CEF-6 (A2 restricted EBV peptides), CEF-7 (A2 restricted CMV peptide), CEF-9 and CEF-10 (A3 restricted flue peptides), and CEF-24 (a B18 restricted CMV peptide). This donor responded in a dominant fashion to two of the predicted peptides, CEF-7 and CEF-11, but did not respond to the six other predicted peptides (highlighted by the arrows). There was no unpredicted response in this donor.

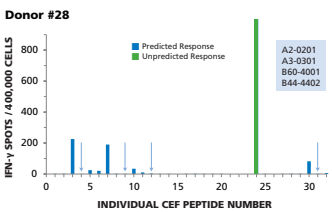

Figure 2: **Example of immune dominance for an unpredicted peptide with magnitude of response higher than predicted dominant responses, while four predicted peptides are not recognized at all.** Based on the HLA-type of this donor, one would expect to detect responses to 11 peptides: CEF-3 and CEF-4 (A2 restricted flu peptides), CEF-5 and CEF-6 (A2 restricted EBV peptides), CEF-7 (A2 restricted CMV peptide), CEF-9 and CEF-10 (A3 restricted flu peptides), CEF-11 and CEF-12 (A3 restricted EBV peptides), CEF-30 (B44 restricted EBV peptide) and CEF-31 (B44 restricted CMV peptide). Of these, two peptides were recognized in a dominant fashion (CEF-3 and CEF-7), two were subdominant (CEF-10 and CEF-30), and responses to the other three were cryptic (CEF-5, CEF-6, and CEF 11). The dominant response was to an unpredicted peptide, CEF 24, originally described as an HLA-B18 restricted CMV peptide, but this donor is B18 negative. CEF-24 apparently can also bind to one of the Class I alleles expressed by this donor. Four predicted peptides were not recognized: CEF-4, CEF-9, CEF-12, and CEF-31 (highlighted by arrows).

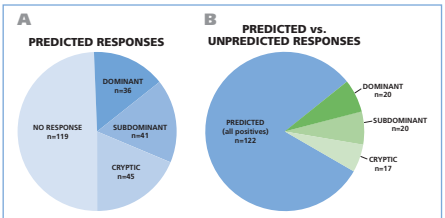

Figure 5: **Predicted vs. actually detected responses for all donors.** Forty-two donors were tested for the 32 individual CEF peptides. (A) Of the predicted 241 responses, in 122 instances responses were detected, and in 119 instances no response was detected. Thus, in 51% of cases (darker blue) the prediction was accurate, and in 49% of cases (lighter blue) it was not. Of the 122 positive responses, 36 were dominant (30%), 41 subdominant (34%), and 45 were cryptic (37%). Of the 241 predicted responses, therefore, only 36 (15%) were dominant. (B) Predicted vs. unpredicted responses among positives. Fifty-seven unpredicted responses (shown in green) were seen vs. the 122 predicted responses. Thus, of all the 179 responses detected, 68% were predicted and 32% were not. Within the unpredicted responses 20 (35%) were dominant, 20 (35%) were subdominant, and 17 (30%) were cryptic. Thus, 70% of nonpredicted responses were prevalent, that is dominant or subdominant.

CEF #	VIRUS	ANTIGEN SOURCE	EPITOPE SEQUENCE	HLA RESTRICTION
CEF-01	Influenza	PB1 (591-599)	VSDGGPNLY	A1
CEF-02	Influenza	NP(44-52)	CTELKLSDY	A1
CEF-03	Influenza	M1 (58-66)	GILGFVFTL	A2
CEF-04	Influenza	PA (46-54)	FMYSDFHFI	A2
CEF-05	EBV	LMP2A (426-434)	CLGGLLTMV	A2
CEF-06	EBV	BMLF1 (259-267)	GLCTLVAML	A2
CEF-07	HCMV	pp65 (495-503)	NLVPMVATV	A2
CEF-08	Influenza	NP (91-99)	KTGGPIYKR	Aw68
CEF-09	Influenza	NP (342-351)	RVLSFIKGTK	A3
CEF-10	Influenza	NP(265-273)	ILRGSVAHK	A3
CEF-11	EBV	BRLF1 (148-156)	RVRAYTYSK	A3
CEF-12	EBV	EBNA 3a (603-611)	RLRAEAQVK	A3
CEF-13	Influenza	M1 (13-21)	SIIPSGPLK	A11
CEF-14	EBV	EBNA 3b (399-408)	AVFDRKSDAK	A11
CEF-15	EBV	EBNA 3b (416-424)	IVTDFSVIK	A11
CEF-16	EBV	BRLF1 (134-143)	ATIGTAMYK	A11
CEF-17	EBV	BRLF1 (28-37)	DYCNVLNKEF	A24
CEF-18	Influenza	NP (418-426)	LPFDKTTVM	B7
CEF-19	EBV	EBNA 3a (379-387)	RPPIFIRRL	B7
CEF-20	Influenza	NP (380-388)	ELRSRYWAI	B8
CEF-21	EBV	BZLF1 (190-197)	RAKFKQLL	B8
CEF-22	EBV	EBNA 3a (325-333)	FLRGRAYGL	B8
CEF-23	EBV	EBNA 3a (158-166)	QAKWRLQTL	B8
CEF-24	HCMV	pp65 (378-389)	SDEEEAIVAYTL	B18
CEF-25	Influenza	NP (383-391)	SRYWAIRTR	B27
CEF-26	Influenza	M1 (128-135)	ASCMGLIY	B27
CEF-27	EBV	EBNA 3c (258-266)	RRIYDLIEL	B27
CEF-28	EBV	EBNA 3a (458-466)	YPLHEQHGM	B35
CEF-29	HCMV	pp65 (123-131)	IPSINVHHY	B35
CEF-30	EBV	EBNA 3c (281-290)	EENLLDFVRF	B44
CEF-31	HCMV	pp65 (511-525)	EFFWDANDIY	B44
CEF-32	HCMV	pp65 (417-426)	TPRVTGGGAM	B7

Table 1: **List of CEF-pool peptides and their restricting MHC Class I alleles.**

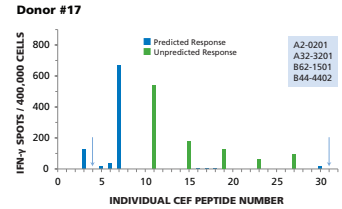

Figure 3: **Immunodominant, subdominant and cryptic responses with both predicted and non-predicted peptides.** In this donor, predicted responses could be expected to 7 peptides: CEF-3 and CEF-4 (A2-restricted flu peptides), CEF-5 and CEF-6 (A2-restricted EBV peptides), CEF-7 (A2-restricted CMV peptide), CEF-30 (B44-restricted EBV peptide) and CEF-31 (B44-restricted CMV peptide). This donor responded to 5 of the 7 predicted peptides: a dominant response was seen to CEF-3 and CEF-7, a subdominant response to CEF-6, and cryptic responses to CEF-5, and CEF-30. This donor produced 5 unpredicted responses: dominant responses to CEF-11, CEF-15 and CEF-19, and subdominant responses to CEF-23 and CEF-27. Two predicted peptides were not recognized: CEF- 4, and CEF-31 (highlighted by arrows).

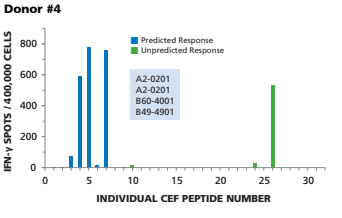

Figure 4: **Only one donor out of the 42 tested responded to all predicted peptides, but also in a co-dominant fashion to an unpredicted one.** This HLA-A2 homozygotic donor responded to all five predicted A2-restricted peptides, mounting co-dominant responses to CEF-4, CEF-5, and CEF-7, a subdominant response to CEF-3 and a cryptic response to CEF-6. While being HLA-B27 negative, this donor showed an unpredicted co-dominant response to CEF-26 that was originally described as an HLA B27-restricted peptide. This unpredicted response was of the same order of magnitude as three predicted responses, and more than 10 times stronger than the predicted responses to CEF-3 and CEF-6. Unpredicted cryptic reactivities were seen to CEF-10 and CEF 24 (originally described as A3- and B18-restricted, respectively).