



CTL Epitope Mapping using Individual Peptide Pools

Christine Siegismund: Robert Koch-Institute, Nordufer 20, 13353 Berlin

Oliver J. Kreuzer: peptides&elephants GmbH, Am Mühlberg 11, 14476 Potsdam, www.peptides.de

A cytotoxic T cell (also known as CTL, T-Killer cells or CD8+ T-cells) belongs to a sub-group of T lymphocytes (a type of white blood cell) that are capable of inducing the death of infected somatic or tumor cells; they kill cells that are infected with viruses or other pathogens. Most cytotoxic T cells express T-cell receptors (TCRs) and a glycoprotein called CD8, which is attracted to non-variable portions of the Class I MHC molecule. That TcR can recognize a specific antigenic peptide (also called epitope in a protein) bound to Class I MHC molecules, present on all nucleated cells. The affinity between CD8 and the MHC molecule keeps the cytotoxic T cell and the target cell bound closely together during antigen-specific activation (Fig 1).

This peptide / epitope is depending on the haplotype of an individual, identifies an infection of certain antigens and the resulting cellular immune response in an individual. Therefore it is very important to know such an epitope of a protein.

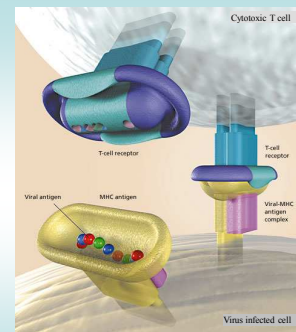


Fig 1: MHC-I - Peptide - TcR complex
Doherty and Zinkernagel, 1996

SIVagm Gag protein, 521 Amino acids in length

103 15mer peptides with an overlap of 10 Amino acids

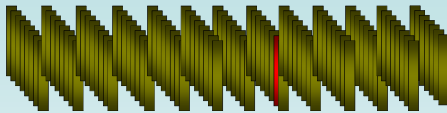


Fig 2: SIVagm Gag protein and the subdivision into peptides

Peptide pools Matrix Format	Pool 11	Pool 12	Pool 13	Pool 14	Pool 15	Pool 16	Pool 17	Pool 18	Pool 19	Pool 20
Pool 1	1	2	3	4	5	6	7	8	9	10
Pool 2	11	12	13	14	15	16	17	18	19	20
Pool 3	21	22	23	24	25	26	27	28	29	30
Pool 4	31	32	33	34	35	36	37	38	39	40
Pool 5	41	42	43	44	45	46	47	48	49	50
Pool 6	51	52	53	54	55	56	57	58	59	60
Pool 7	61	62	63	64	65	66	67	68	69	70
Pool 8	71	72	73	74	75	76	77	78	79	80
Pool 9	81	82	83	84	85	86	87	88	89	90
Pool 10	91	92	93	94	95	96	97	98	99	100

Fig 3: Composition of the peptide pools following the matrix format

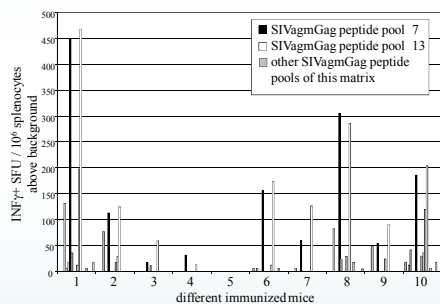


Fig 4: T cell based ELISpot assay

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Fig 5: Detection of the single peptide / epitope of SIVagm Gag protein in mice with the haplotype H-2K^b

Peptide 63: DRFYKAIRAEQASGE

CTL epitope mapping of proteins from different species with the peptide libraries from **peptides&elephants** (LIPS®) is quite easy and works perfect. The protein is divided into peptides with a length of 15 amino acids with an overlap of 10 amino acids. The peptides are provided lyophilized in 96 deep well plates, each well contains a single peptide. Individual pools are generated, in the way that each peptide is present in two pools. The pools are tested and the obtained results are plotted in the matrix format. The matrix is set up in the way that each peptide occurs once on the ordinate and once on the abscissa.

The positive peptide is identified via a positive (X/Y) signal pair in the matrix. Single positive spots can be excluded as false positive results.

In a second experiment the revealed peptide can be picked out of the library and the assay results can be confirmed with this single peptide.

This is the big advantage of the individual created peptide pools compared to Ready-to-use overlapping peptide pools spanning the whole protein. With these previously pooled peptides it is not possible to build this individual matrix and detect single CTL epitopes. Individual peptides provided by **peptides&elephants** enable the establishment of the matrix. With this, individual peptides can be tested for CTL responses. The deconvolution of the matrix therefore allows mapping of single CTL epitopes.

Enzyme-linked immunospot (ELISpot) assay:

All ELISpot assays were performed using RPMI-1640 medium containing 10 % FCS, 1 % L-Glu, 100 U/ml Pen/Strep and 100 µg/ml neomycin. The ELISpot plates were pre-coated with anti-mouse INF γ -antibody mAK AN18 (Mabtech AB, Sweden). The Assays were performed using the Mabtech-protocol and fresh isolated splenocytes or rapidly thawed cells from the liquid nitrogen with subsequent regeneration of 2 hrs in the CO $_2$ incubator. 2x10⁵ cells were stimulated with the final concentration of 5 µg/ml Gag peptide pool (peptides&elephants, Germany), 5 µg/ml nonsense peptide (peptides&elephants, Germany) or 2 µg/ml pokeweed mitogen (PWM; Sigma, Germany) co-stimulated with 30 U/ml muII-2 for 16 hrs at 37°C in a 5 % CO $_2$ incubator. Cells responding to stimulation by production of INF γ were detected with the anti-mouse INF γ -antibody mAK R4-6A2-Biotin (Mabtech AB, Sweden). The spots were developed with the colour reagent 5-bromo-4-chloro-3-indolyl phosphate / nitro blue tetrazolium and enumerated using an ELISpot-Reader. In the screening assay the Gag peptide pools (matrix format) were tested in single measurements (Fig 4). After matrix deconvolution (Fig 5) most of the individual peptides were screened in triplicates to confirm the response. The frequency of peptide-specific T-cells is expressed as the number of INF γ -secreting cells per 10⁶ splenocytes. Positive responses were defined as those where the amount of ELISpots are above the mean value + 3xSD of the in triplicates tested nonsense peptide.

To achieve the optimal peptide with 8-10 amino acids the fine mapping of this 15mer should be the next step.