Truncated Peptide Libraries for Cytotoxic T-Cell Epitope Mapping

Libraries of truncated peptides are powerful tools for identifying the peptides recognized by T-cells

Topics in this article:

Cytotoxic T lymphocytes (CTL) recognize an epitope comprised of a self portion (Class I MHC) and a foreign portion (a short peptide). It is known that the foreign peptide part of CTL epitopes consists of peptides using between 8 and 11 residues in length [1]. See Figure 3 for a diagramatic illustration of the binding of a 9-mer to a Class I MHC molecule.

Previous epitope mapping strategies for CTL using synthetic peptides were imprecise and relied on a breakdown process acting on the peptides, either in the culture medium or in the cells, to generate the active peptides from longer sequences[2,3]. Another approach, use of predictive methods, is effective at locating substantial numbers of CTL epitopes when the binding motifs of all the Class I MHC isotypes and allotypes are known, but even then there can be epitopes which do not conform exactly to consensus motifs, or which vary in length. To do this with conventional peptide synthesizers is costly and impractical because of the large number of peptides and the large amount of testing required.

Improved Strategies for Epitope Mapping

PepSets™ Peptide Libraries are powerful tools for identifying the peptides recognized by T-cells. Mapping cytotoxic T-cell (CTL or T_C) epitopes of a whole protein can be difficult because of the large number of possible active sequences, and the unknown length requirements for MHC binding and bioactivity (whether 8, 9, 10 or 11 residue peptides).

This article presents the use of a novel technology for the synthesis and testing of CTL epitopes using PepSets Peptide Libraries in which each nominal 11-mer peptide is made up of an equimolar mixture of the four C-terminal peptides comprising the 8-mer to 11-mer peptides. Because the library contains all the possible active CTL epitopes, it is more effective than longer peptides for mapping cytotoxic T-cells and gives a more precise answer from the initial CTL epitope screening experiments.

The Truncated PepSets Peptide Library PepSets-T_C overcomes previous limitations by allowing the researcher to test all possible peptides homologous with the protein of interest, with lengths between 8 and 11 residues.

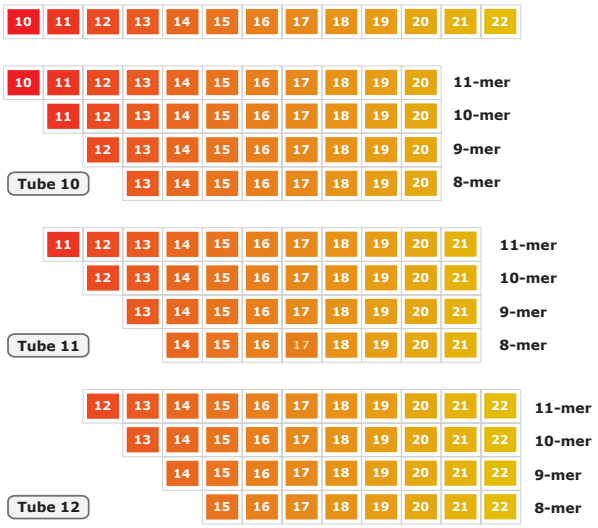

Figure 1: Portion of a protein sequence, residues No. 10 to 22

Methods

Mimotopes' proprietary technology has been applied to the PepSets Peptide Library and devised a method of synthesizing, in one tube, an equimolar mixture of peptides of any series of lengths, provided they have a shared C-terminal sequence.

By making the required number of peptide tubes to span the sequence (around the same as the number of residues in the protein), all possible 8, 9, 10 and 11-mer peptides of the protein are present in similar quantity. These peptides can be further pooled for assay, knowing that because all potential epitopes are present, all epitopes will be efficiently detected, even at very low added doses of peptide or in the

presence of other peptides. See Figure 1 for a diagrammatic representation of the composition of each tube for such a CTL mapping set.

To compare the effectiveness of a Truncated PepSet against the effectiveness of a conventional set of peptides, the overlapping peptides corresponding to the F (fusion) protein of Respiratory Syncytial virus (RSV), a protein of 574 AA residues, were made in two formats. The first format was a set of overlapping peptides in which each tube contained a single 14-mer peptide. The second format was a set of overlapping truncated peptides in which each tube contained the 8-mer, 9-mer and 10-mer sequences. These two sets were compared for effectiveness at detection of cytotoxic T-cells in ELISPOT using human peripheral blood mononuclear cell preparations.

Results

The peptide mixtures can be used directly in gamma interferon ELISPOT, an assay for CTL activation. In this early study, the Truncated PepSet was a set of 565 10-mers (overlap of 9 residues) rather than 11-mers with an overlap of 10 residues. Each nominal 10-mer contained the 9-mer and 8-mer, and it was compared with a set of 188 non-truncated 14-mer peptides with an overlap of 11 residues.

The sixteen subjects were healthy volunteers known to be immune to RSV. Table 1 shows the comparison of the frequency of positive ELISPOT data summed by peptide and donor, i.e. each instance of a positive ELISPOT reading with a donor and a peptide preparation was scored as 1, and summed to give the totals shown. It can be seen that, as expected, there were many more positive peptide/subject combinations from the truncated peptides than from the single 14-mers.

This is expected because two successive 10-mer peptides can easily span a single CTL epitope, for example if it were a 9-mer, then potentially, two contiguous overlapping 10-mers could be stimulatory. More significant however is the observation that the truncated 10-mers revealed 35 unique stimulatory sites along the F protein sequence, many more than the 14-mers. This is despite the possibility that at least some of the positive data from the 14-mers may have been due to T_h (T helper) cells, whereas the ELISPOT with the truncated 10-mers almost certainly is due to T_c because 10-mers have been generally found to be too short to act as effective T_h epitopes.

Subsequent work [4] has shown that a strongly recognized peptide from this protein, sequence RELPRFMNYT, is a frequently recognized CTL epitope seen in the context of the HLA A*0101 MHC Class I allotype. Significantly, this epitope was not detected in initial screens with the 14-mer peptides spanning this sequence. The higher frequency of detection of ELISPOT positive peptides with PepSet-T_c peptides is a clear indication that the truncated 10-mers are more efficient at priming antigen-presenting cells with externally added

Peptide Length	No. positive subject/ peptide combinations	No.unique ELISPOT combinations	No.positive peptide preparations
10-mers (truncated)	105	35	59
14-mers (single)	35	17	25

Table 1: Comparison of Two Peptide Library Designs for Scanning of Human PBMC for T_c Epitopes by IFN-gamma ELISPOT

Peptides of the RSV Fusion Protein, Total 574 Amino Acids, 16 Subjects, 565 Truncated 10-mer peptides, 188 Non-truncated (single) 14-mers

peptide than the 14-mers, and thus are a more effective reagent for discovering biologically signficant T_c epitopes.

Figure 2 shows the analytical LC profile of the peptides in one tube of a truncated peptide containing the 8-mer, 9-mer, 10-mer and 11-mer of the sequence of interest. It is seen that there are four major peaks, corresponding to the full length sequence and the three truncated versions of the full length peptide.

Discussion

Further pooling of truncated peptide mixtures can reduce the amount of screening work needed [5]. When stimulatory pools are found, the active peptide mixture can be deduced by testing the individual samples which had been used to prepare the stimulatory pool. It has been shown that mixing of CTL epitope peptides does not cause masking of the active sequences [5]. Strategies for pooling and the advantages of doing so have been explained in some detail [6]. In another recent study with truncated peptides [7], it was confirmed that there is a single CTL epitope in the E7 oncoprotein of human papillomavirus type 16. This study also included the use of a combination of phosphorylated and non-phosphorylated amino acids, necessary because the protein is known to be at least partly phosphorylated *in vivo*.

Conclusion

Sets of short, deliberately truncated peptides are much more effective for mapping T_c epitopes than longer single peptides spanning the same sequence. This is expected on theoretical grounds and was confirmed in an initial screening study.

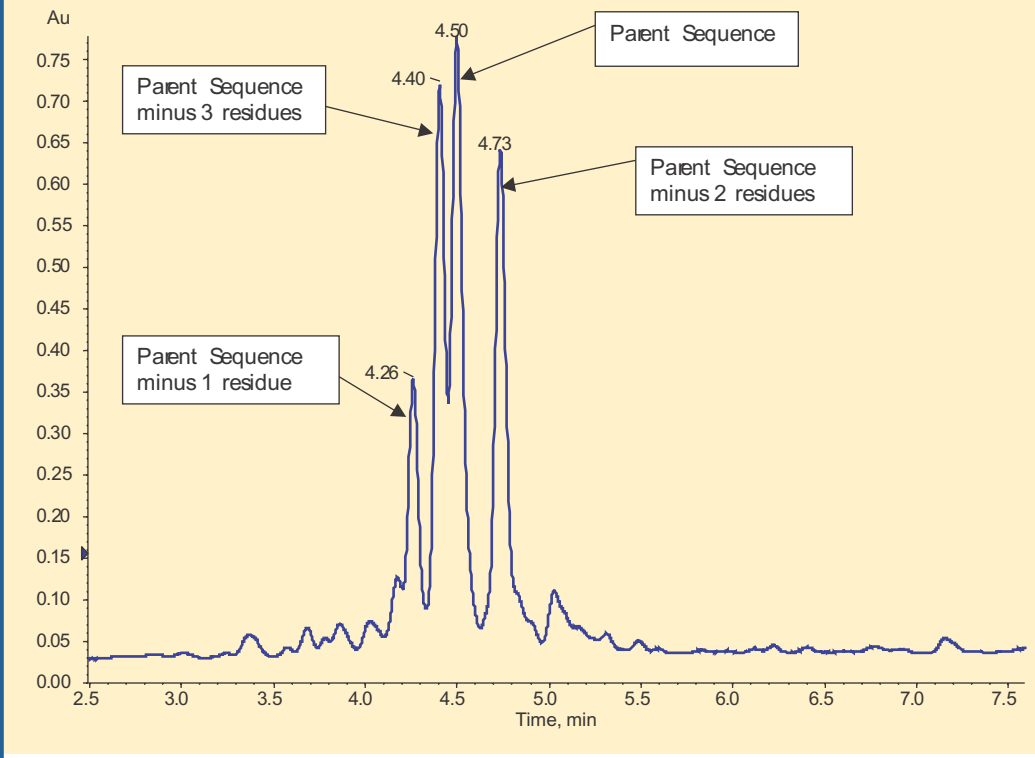

Figure 2: LC Profile of a Four-peptide Mixture Synthesized using Truncation Technology

Each peak was confirmed by MS as comprising one of the four target sequences

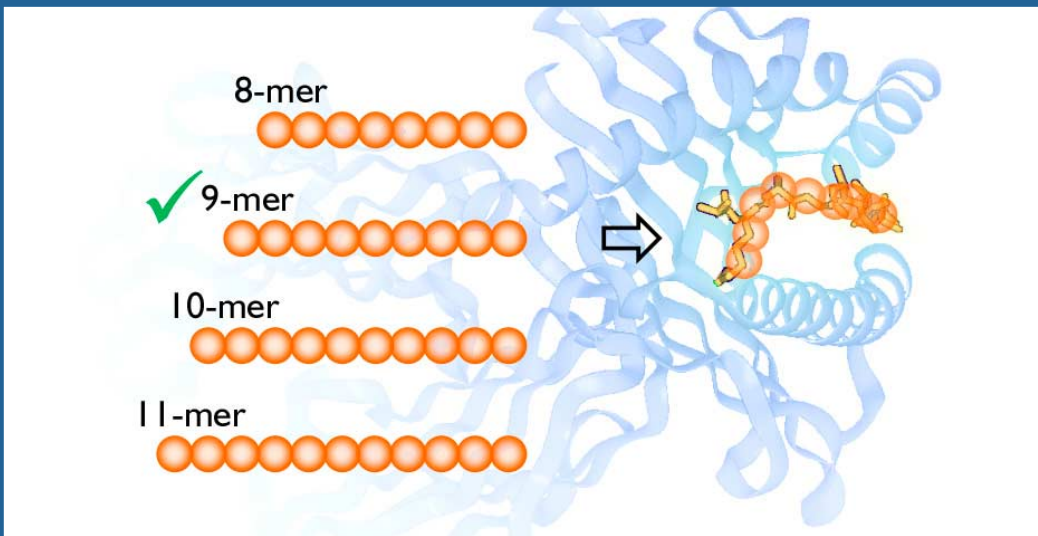

Figure 3: Peptide Bound to Class I MHC

Only a peptide of the appropriate length, specifically a 9-mer in this case, will bind to the MHC molecule. PepSets™-T_C are an ideal way to perform screening with a wide range of peptides for major Histocompatability Antigen binding or T-cell activation

Applications in Biological Research

Some of the most frequently used applications are listed below.

- **Immunology**
  The most common use of PepSets in immunology is for T and B cell epitope mapping.

- **Pharmacology/Physiology**
  The most common use of PepSets in pharmacology/physiology is for locating or analoging of pharmacologically active peptides such as neurotransmitters, hormones or chemokines.

- **Biochemistry**
  PepSets are used in biochemical studies as enzyme substrates, inhibitors, or ligands.

- **Molecular Biology**
  Peptide libraries can be used to look for the sites of protein-protein or protein-nucleic acid interaction. Testing can be by direct capture of the protein or nucleic acid, by measurement of captured peptide, or by inhibition of another known binding interaction. Protein sequences can be screened for interaction sites by making panels of antipeptide antibodies covering the whole protein.

- **Immunodiagnostic Test and Vaccine Development**
  Preliminary screening of all possible sites for effectiveness of antipeptide antibodies in a disease model can lead directly to the best vaccine candidates and save years of expensive "trial and error" research based on predictive methods. Likewise, immunodiagnostic tests need to use peptide which is recognized most frequently and strongly by immune sera or cells.

- **Medical and Veterinary Clinical Immunology Studies**
  Understanding the basis of clinical disease related to the immune system, whether arising from protective immunity, immunopathology, transplant rejection or reaction to mutated cancer cells may require testing of large numbers of individuals over many proteins or epitopes. This may only be possible with peptide libraries, and to achieve the broad surveys needed, it is critical to use peptide reagents with the right characteristics. When epitopes become defined through the use of libraries, frequently the next step is to work with purified peptides. Mimotopes purified custom peptide products can be supplied to a quantity and purity specification suitable for most projects and budgets.

- **New Drug Discovery**
  Peptide Libraries can be a rich source of candidates for a drug development program. For example, an SAR study on a bioactive peptide fragment can reveal the critical residue(s) for bioactivity, which can then be further "analoged" to obtain more potent leads and peptidomimetics.

References

1. Rammensee, H.G., Friede, T. and Stevanovic, S. (1995) MHC ligands and peptide motifs: first listing. Immunogenetics 41, 178.

2. Luckey, C.J., King, G.M., Marto, J.A., Venketeswaran, S., Maier, B.F., Crotzer, V.L., Colella, T.A., Shabanowitz, J., Hunt, D.F. and Engelhard, V.H. (1998) Proteasomes can either generate or destroy MHC class I epitopes: evidence for nonproteasomal epitope generation in the cytosol. J. Immunol. 161, 112.

3. Maecker, H.T., Dunn, H.S., Suni, M.A., Khatamzas, E., Pitcher, C.J., Bunde, T., Persaud, N., Trigona, W., Fu, T., Sinclair, E., Bredt, B.M., McCune, J.M., Maino, V.C., Kern, F. and Picker, L.J. (2001) Use of overlapping peptide mixtures as antigens for cytokine flow cytometry. J. Immunol. Methods 255, 27.

4. Rock, M.T. and Crowe, J.E. (2003) Identification of a novel human leucocyte antigen-A*01-restricted cytotoxic T-lymphocyte epitope in the respiratory syncytial virus fusion protein. Immunology 108, 474.

5. Sun Y., Iglesias E., Samri A., Kamkamidze G., Decoville T., Carcelain G. and Autran B. (2003) A systematic comparison of methods to measure HIV-1 specific CD8 T cells. J. Immunol. Methods 272, 23.

6. Roederer M. and Koup, R.A. (2003) Optimized determination of T cell epitope responses. J. Immunol. Methods 274, 221.

7. Khammanivong, V., Liu, X.S., Liu, W.J., Rodda, S.J., Leggatt, G.R., Tindle, R.W., Frazer, I.H. and Fernando, G.J.P. (2003) Paucity of functional CTL epitopes in the E7 oncoprotein of cervical cancer associated human papillomavirus type 16. Immunol. Cell Biol. 81, 1.

Mimotopes International
11 Duerdin St, Clayton
VIC, Australia 3162
service@mimotopes.com
www.mimotopes.com

Mimotopes Asia Pacific
Tel: +61 3 9565 1111
Fax: +61 3 9565 1199
australia@mimotopes.com

Mimotopes Europe
Tel: +44 870 460 1500
Fax: +44 870 460 1501
europe@mimotopes.com

Mimotopes US East
Tel: +1800 633 8161
Fax: +1800 424 3970
useast@mimotopes.com

Mimotopes US West
Tel: +1800 644 1866
Fax: +1800 655 1866
uswest@mimotopes.com