Peptide Synthesis Services at Mimotopes: from Vaccine to Drug Development and then back again

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Since the mid 1980's, our group has been performing multiple solid phase synthesis of both peptide and small molecule compounds on grafted surfaces (SynPhase™ Crowns and Lanterns (Multipin™ synthesis))[1-3]. The Multipin synthesis approach originated as an immunological tool for epitope mapping, but has since diversified for use in molecular immunology and drug discovery. The fundamental advantage of this technology, which is an alternative to beaded cross-linked resins, is the ease of handling large numbers in multiple parallel syntheses. The presentation will focus on utilizing the Multipin method of peptide synthesis for the following applications, focusing for the Institute for Animal Health on the immunology/vaccine applications:

- General epitope mapping for applications in vaccine design and research including applications for both linear and discontinuous epitope mapping
- The synthesis and use of a broad-spectrum fluorescence-based peptide library for the rapid identification of protease substrates[4]
- The synthesis of small constrained cyclic peptides for drug discovery applications[5]
- High-throughput screening of cytotoxic T-cell epitopes for vaccine development

Various approaches are currently proposed to develop therapies for the prevention and treatment of infectious diseases and of cancer. One of the most promising approaches is the development of vaccines that elicit a cytotoxic T lymphocyte response. Considering the myriad of possible peptide sequences, coupled with the genetic variability of MHC molecules among individuals, the challenge of selecting the "right" target antigen or epitope combination (whether 8, 9, 10 or 11 residue peptides) presents a major obstacle in the development of cell-mediated vaccines. In approaching the problem of *in vitro* discovery and evaluation of potential antigen-specific epitopes, the presentation will present the use of a new technology for peptide synthesis and high throughput screening of CTL epitopes. Scientists from Pure Protein have developed a state-of-the-art peptide competition assay that is applied in 2 tiers. Tier 1 is the high-throughput screening of hundreds-to-thousands of overlapping peptides to identify candidate peptide epitopes (PolyScreen) with any affinity for a class I MHC molecule. Peptides of affinity are then detailed for their precise strength of interaction in tier 2 (PolyTest). This 2-tiered approach allows the rapid identification of potential peptide candidates (synthesized as "truncated overlap Pepsets™" by Mimotopes) followed by fine characterization of these candidates. Because the peptide library contains all the final active CTL epitopes, it is more effective than cell-based screening with longer peptides (which may or may not be processed into CTL epitopes) and it provides a more precise epitope answer as compared to CTL epitope screening experiments. Developed onto a high-throughput screening platform, the new process bridges current screening gaps and considerably shortens the route to clinical development by allowing a systematic ranking of candidate epitopes for subsequent functional studies.

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4. Thomas, D.A., Francis, P., Smith, C., Ratcliffe, S., Ede, N.J., Kay, C., Wayne, G., Martin, S.L., Moore, K., Amour, A., and Hooper, N.M., A broad-based fluorescence-based peptide library for the rapid identification of protease substrates. *Proteomics*, **2006**, (6), 2112-2120
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