

Qualification and Use of Peptide Libraries for Clinical Trial Immunomonitoring

J. H. Cox and P. Hayes

International AIDS Vaccine Initiative Human Immunology Laboratory, Chelsea & Westminster Hospital, London

Understanding cellular immune responses, and in particular T cell responses, is critical for understanding the pathogenesis of disease and for vaccine development. Interrogating T cell responses with libraries of overlapping peptides has become a standard practice. A large component of the laboratory budget can be spent on peptides and here we share some common practices for assessing the quality and specificity of peptides for use in T cell assays.

Introduction

Synthetic peptides are used for the stimulation of peripheral blood mononuclear cells (PBMC) or cells derived from mucosal and other tissues in ELISPOT, intracellular cytokine staining (ICS) and other immunological assays to assess vaccine induced or other immunological responses (1-6). The synthetic peptides are designed to precisely match the vaccine insert sequence, the pathogen or tumor of interest or can be specifically designed to capture variant sequences (7). Synthetic peptides are typically ordered as individual peptides of 9-20 amino acids (AA) in length with the most common format of 15 AA (15-mers) with overlapping sequences of 11 AA in length. They are synthesized in bulk quantities by manufacturers specializing in custom peptide synthesis. The peptides are synthesized to a desired purity and specification; at least 70% purity for research purposes and 90% purity for clinical trial applications. Each manufacturer provides information on the biochemical characterization of the peptides by stand-alone Mass Spectrometry (MS), High Performance Liquid Chromatography (HPLC) or HPLC-MS combinations. In addition, peptide content determination by amino acid analysis (AAA) provides the basis for accurate determination of final peptide concentrations.

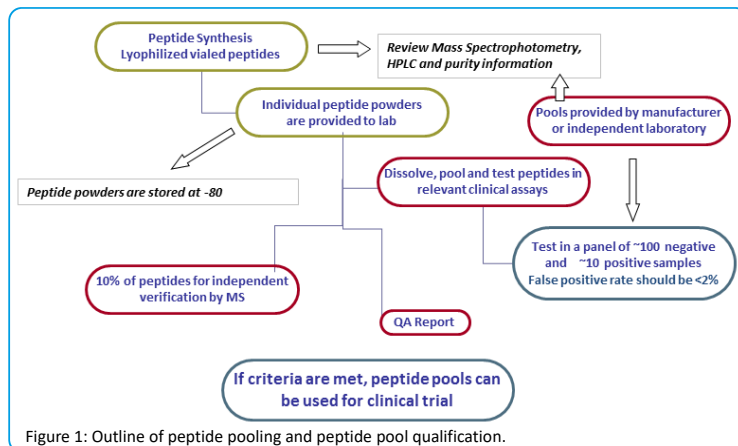
hundreds of PBMC samples to get coverage of epitopes across multiple HLA alleles. This combined with the complexity of assessing epitope specific T cell responses in sensitive and specific T cell assays would make this task virtually impossible. However, the presence of individual peptides in peptide mixtures of limited complexity can be determined by validated, high-performance LC-MS methods. In addition, definition and tracking of “marker peptides” with unique HPLC retention times and molecular weights within such pools allow GLP-compliant preparation of mixtures containing even hundreds of peptides. The lyophilized (powdered) synthetic peptides are typically dissolved in dimethyl sulfoxide (DMSO) and either stored in individual aliquots or pooled and further aliquoted. The peptide pools are then tested in ELISPOT, ICS, CFSE or other appropriate clinical immunology assays using PBMC from donors with no anticipated response to the peptide pools of interest to assess the false positive response rate and PBMC from donors who would be expected to respond to the peptide pools. In practice, it is not always easy to obtain appropriate positive and negative responders, for example in endemic malaria countries, there may be low T cell responses in exposed individuals making it difficult to separate true positives from negatives. We share here an example of the biological qualification of a set of HIV peptide pools that were synthesized and pooled by JPT Peptide Technologies.

Materials & Methods

A plan for qualifying peptides and peptide pools is outlined in **Figure 1**. Approximately 700 15-mer peptides with an 11-mer overlap were made to 90% purity representing HIV-1 Gag, Pol, Nef, Tat, Vif and Env peptides (**Figure 2 inset**). The peptides were tested against 95 HIV-seronegative and 10 HIV-seropositive frozen PBMC samples using an Interferon-gamma (IFN- γ) ELISPOT assay in a GCLP-accredited laboratory (13, 14). The PBMC from HIV seronegative individuals were representative of the populations that would be tested in vaccine clinical trials; 38 from North America, 36 from South Africa and 21 from East Africa. The PBMC viability was > 97% overall and all individuals had a PHA response. The results are expressed as background-subtracted spot forming cells / million (SFC/m) PBMC. A positive response is defined as the number of SFC/m and had to satisfy the following criteria: 1) Average number of background-subtracted spots >38 SFC/m; 2) Mean count must be >4 times mean background; 3) Mean background must be <50 SFC/m PBMC.

Results

Figure 2 left panel shows the cumulative distribution of the ELISPOT responses with the inserted table showing the basic statistics for each peptide pool. **Figure 2** right panel shows the



This report outlines the recommendations for defining the biological quality of peptides and for pooling individual peptides into pools of desired size for use in cellular immune response assays (8-10). False positive signals may arise from 1) impurities or contaminations of the peptides, 2) authentic biological stimulation as a result of cross-reactive T cell receptor or HLA degeneracy, 3) competition because of too many peptides per pool or peptides at a high concentration, or 4) sub-optimal length of peptides (3, 10-12). To assess whether each individual peptide in a peptide pool is capable of stimulating T cell responses would require testing of

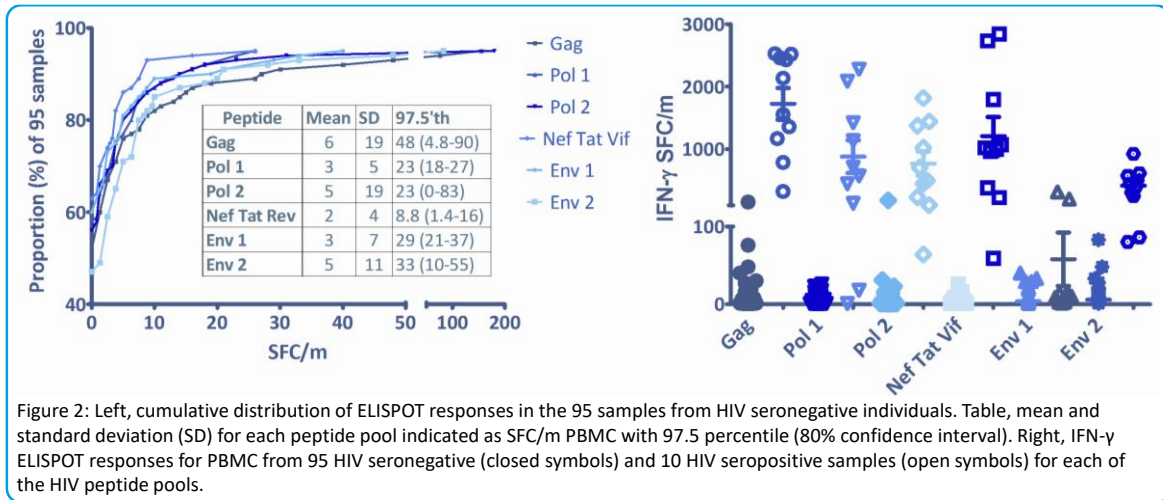


Figure 2: Left, cumulative distribution of ELISPOT responses in the 95 samples from HIV seronegative individuals. Table, mean and standard deviation (SD) for each peptide pool indicated as SFC/m PBMC with 97.5 percentile (80% confidence interval). Right, IFN-γ ELISPOT responses for PBMC from 95 HIV seronegative (closed symbols) and 10 HIV seropositive samples (open symbols) for each of the HIV peptide pools.

IFN-γ ELISPOT responses for PBMC from 95 HIV seronegative and 10 HIV seropositive samples for each of the HIV peptide pools. None of the seronegative samples were positive by the pre-defined criteria and therefore this allowed the determination of an ELISPOT positivity cut-off for the clinical trial which was set at 38 SFC/m. So far out of 69 baseline samples from the clinical trial there has been only 1 false positive response to Env P1 (false positive rate = 1.4%).

Discussion & Conclusions

Before starting a clinical trial, it is very important to characterize the peptide pools that will be used to ascertain whether there is stimulation of T cell function where T cell responses would not be expected by use of PBMC samples from subjects who have not been exposed to the pathogen under study. Likewise the ability to stimulate T cell responses where a response is expected should be demonstrated by use of PBMC samples from subjects who have been exposed and infected with the pathogen under study. The results of this particular study showed that high quality peptides induced minimal false positive responses in an IFN-γ ELISPOT either during the peptide qualification or in samples collected prior to vaccination.

Acknowledgments

We'd like to thank the IAVI Human Immunology Laboratory members in London and to our laboratory partners in the HIV vaccine field (CAVD, HVTN, NVITAL and MHRP) for learning together the pitfalls in assay development and dedication to developing an effective HIV vaccine.

References

- "Comprehensive Epitope Analysis of Human Immunodeficiency Virus Type 1 (HIV-1)-Specific T-Cell Responses Directed against the Entire Expressed HIV-1 Genome Demonstrate Broadly Directed Responses, but No Correlation to Viral Load" Addo et al., J. Virol. (2003)
- "Analysis of Total Human Immunodeficiency Virus (HIV)-Specific CD4+ and CD8+ T-Cell Responses: Relationship to Viral Load in Untreated HIV Infection" Betts et al., J. Virol. (2001)
- "Detection of HIV vaccine-induced cell-mediated immunity in HIV-seronegative clinical trial participants using an optimized and validated enzyme-linked immunospot assay" Dubey et al., J Acquir Immune Defic Syndr (2007)
- "T-cell epitope mapping by flow cytometry" Kern et al., Nat Med (1998)
- "Revival of the identification of cytotoxic T-lymphocyte epitopes for immunological diagnosis, therapy and vaccine development" Liu et al., Exp. Biol. Med. (Maywood, N.J.) (2011)
- "Use of overlapping peptide mixtures as antigens for cytokine flow cytometry" Maecker et al., J Immunol Methods (2001)
- "Peptide selection for human immunodeficiency virus type 1 CTL-based vaccine evaluation" Li et al., Vaccine (2006)

- "HIV Type 1 Vaccines for Worldwide Use: Predicting In-Clade and Cross-Clade Breadth of Immune Responses" Finnefrock et al., AIDS Res Hum Retroviruses (2007)
- "Optimized determination of T cell epitope responses" Roederer & Koup, Journal of Immunological Methods (2003)
- "Moving to HIV-1 Vaccine Efficacy Trials: Defining T cell Responses as Potential Correlates of Immunity" Russell et al., J Infect Dis (2003)
- "Peptide impurities in commercial synthetic peptides and the implications for vaccine trial assessment" Currier et al., J Virol (2007)
- "Effect of peptide pools on effector functions of antigen-specific CD8+ T cells" Suneetha et al., J Immunol Methods (2009)
- "Concordant Proficiency in Measurement of T-Cell Immunity in Human Immunodeficiency Virus Vaccine Clinical Trials by Peripheral Blood Mononuclear Cell and Enzyme-Linked Immunospot Assays in Laboratories from Three Continents" Boaz et al., Clin Vaccine Immunol (2009)
- "Equivalence of ELISPOT Assays Demonstrated between Major HIV Network Laboratories" Gill et al., PLoS ONE (2010)

The Author



Josephine H Cox
 jcox@iavi.org
 International AIDS Vaccine Initiative
 Human Immunology Laboratory
 Chelsea & Westminster Hospital
 London

Josephine H Cox is the Director of Clinical Immunology for the International AIDS Vaccine Initiative (IAVI), she works with the Human Immunology Laboratory (HIL) at Imperial College London. She got her PhD in Immunology at Manchester University, UK before settling in the US. She has helped set up cellular immunology laboratories in Thailand and Africa ready for phase I-II HIV vaccine trials as well as studies on the immunopathology of HIV disease.

The Company

JPT Peptide Technologies is an ISO 9001:2015 certified provider of innovative peptide solutions for: cellular and humoral immune monitoring, seromarker discovery & validation, vaccine target discovery, peptide lead identification & optimization, targeted proteomics, and enzyme profiling.

Contact us for further information!

email: peptide@jpt.com

Please visit us online at:

<https://www.jpt.com/>