A Modular Approach for Epitope Discovery and High-Resolution Profiling of Humoral Immune Responses

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Introduction

An individual's antibody repertoire is an imprint of previous infections and potential immunity to self antigens. Changes in this immune imprint might presage disease onset and progression. This is clearly of relevance for infectious disease and also for cancer. Detailed knowledge of the individual repertoire is not only useful for the diagnosis of diseases but also for monitoring the success of therapeutic interventions. Here we present a comprehensive and modular three-step antibody epitope and seromarker profiling workflow (Figure 1). First, multiplexed epitope discovery is carried out for candidate proteins. Using High Content Peptide Microarrays (Figure 2, left panel), thousands of peptides are screened. Next, the hits are confirmed by selective antigen profiling with multiple serum samples. Applying Multiwell Peptide Microarrays (Figure 3, left panel), thousands of samples can be tested. Finally, the biomarkers are validated by Peptide ELISA.

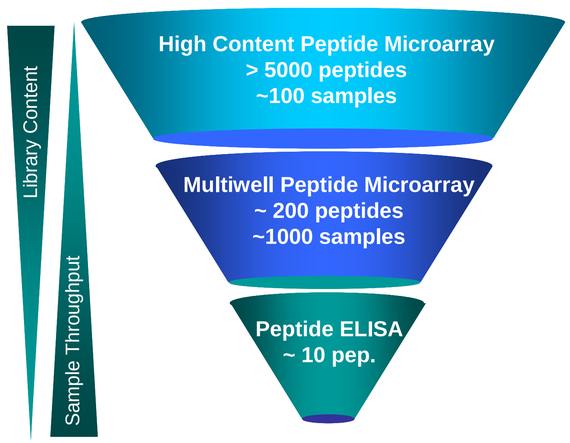

Figure 1: Biomarker Discovery Workflow. (1) Multiplexed Epitope Discovery using High Content Peptide Microarrays. (2) Selective Antigen Profiling using Multiwell Peptide Microarrays. (3) Marker Validation using Peptide ELISA.

Application Example

Nearly all adult humans are infected by Epstein-Barr virus (EBV). Mostly, the infection persists lifelong without being recognized. Using High Content Peptide Microarrays, we investigated the B-cell immune response to EBV. Four humans are shown to be infected by EBV and having both, common and individual reactivity patterns to EBV antigens (Figure 2, right panel). We discovered a remarkable reactivity pattern for the EBNA1 antigen. Using Multiwell Peptide Microarray, the pattern was confirmed (Figure 3, middle panel). Two peptides were selected for further investigation (Figure 3, right panel). Reactivities observed on microarrays were verified by Peptide ELISA (Figure 4, left panel). Eleven additional humans were analyzed by Peptide ELISA. Consistent with the high prevalence of EBV, ten humans were found to be positive (Figure 4, right panel).

Multiplexed Epitope Discovery

A limited number of samples (typically 20 to 200) are profiled on PepStar™ High Content Peptide Microarrays displaying up to 6912 peptides in triplicates. This allows the coverage of a broad range of known and potentially relevant cancer antigens as well as antigens from infectious agents. The low cost per peptide and high peptide throughput allow selection of relevant peptides from thousands of candidate peptides.

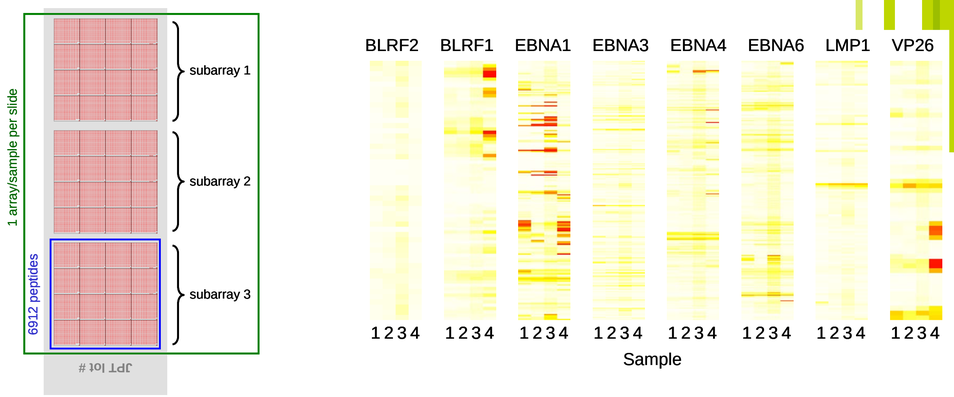

Figure 2: High Content Peptide Microarrays (left panel) were used for investigation of the antibody repertoire in four human blood samples to eight EBV antigens (right panel).

Selective Antigen Profiling

Pre-selected antigens and peptides will be tested against a large number of samples (typically 200 to 1000). For this purpose PepStar™ Multiwell Peptide Microarrays will be used taking advantage of parallel and economic testing of 21 patient samples on a single microarray. The format is limited to 192 candidate peptides (in triplicate) per sample allowing systematic profiling of candidate peptides with large numbers of test samples. The low cost per sample and high sample throughput allow verification of candidate peptides for a significant number of samples.

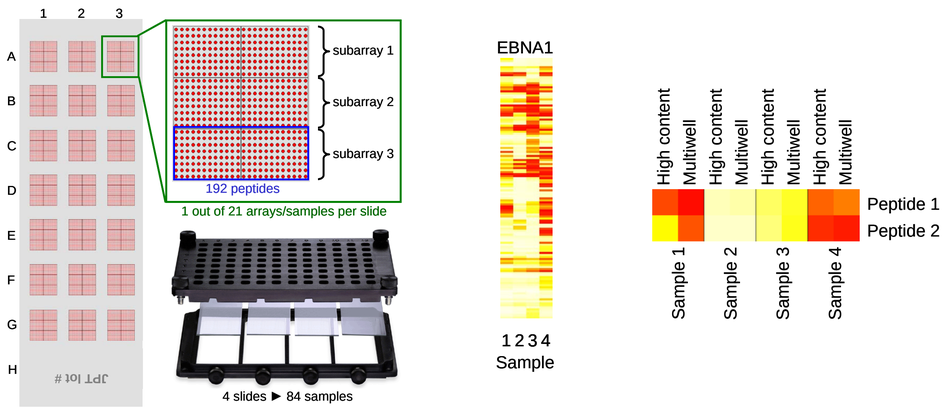

Figure 3: One antigen was selected for further investigation. The samples were analyzed using one Multiwell Peptide Microarray (left panel). Matching reactivity patterns were found for both array types (middle panel). Two peptides were selected (right panel).

Marker Validation

A robust Peptide ELISA platform was developed based on highly purified peptides allowing both, the validation of the selected candidates as well as detailed analysis of the recognition. The platform enables transfer of results towards diagnostic assays and the selection of vaccine candidates. Peptide ELISA is a reliable, economic and common assay.

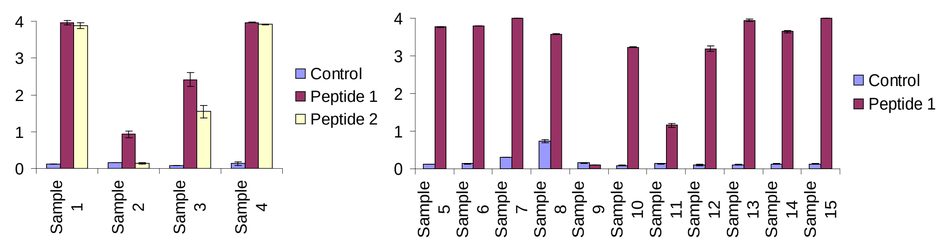

Figure 4: Peptide ELISA was applied for validation of the results (left panel). Using Peptide ELISA, relevance of selected peptide was confirmed for additional samples (right panel).

A platform of complementary assay formats enables the identification and validation of biomarkers in diverse indications for many uses:

- Cancer
- Infectious diseases
- Autoimmune diseases
- Allergies
- Clinical trials
- Vaccination studies
- Companion diagnostic
- Patient stratification

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