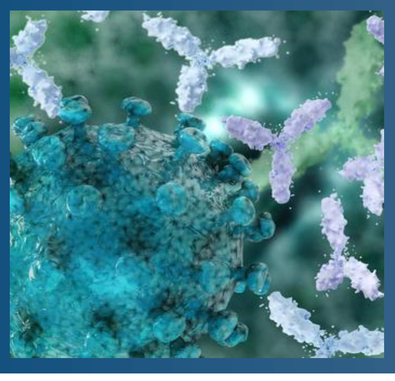

High Density Peptide Microarrays: An Essential Tool for Comprehensive Biomarker Discovery and Virus Epitope Mapping

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Abstract

Introduction

Serological diagnosis of viral diseases often remains challenging either due to cross-reactivity towards protein antigens, heterogeneous immune responses or poorly defined antigenic properties of proteins used in serological assays. Furthermore, standardization of the quality of these proteins can be difficult. Particularly, results of Hepatitis E Virus (HEV) serological assays vary considerably due to differences in the antigen content. In contrast, serological assays based on carefully selected peptides are ideally suited to overcome these problems.

Objectives

The precise knowledge of antigenic properties of proteins and their underlying epitopes could provide the basis for an innovative, multiplexed serological assay with a higher sensitivity and specificity.

Material and Methods

High density peptide microarrays can display large numbers of putative target proteins translated into overlapping peptides. Antibody responses against linear and conformational epitopes can be analyzed with unmet speed and precision to yield high and low immunogenic IgG-, IgA- and IgM-specific marker epitopes.

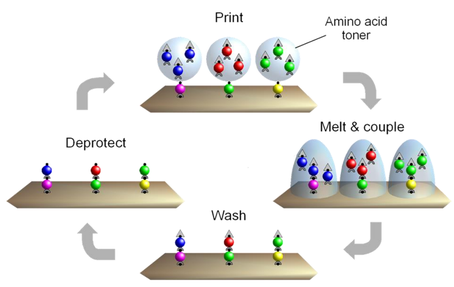
Results

As an example, we chose HEV to conduct a proteome-wide epitope mapping in order to find new diagnostically relevant peptide biomarkers. Therefore, the proteomes of prototype strains of all human pathogenic genotypes of HEV (genotype 1, 2, 3, 4 and 7) were translated into linear or cyclic peptides and synthesized on a single peptide microarray. Linear and conformational epitope mappings for IgG were performed using 6 IgG-positive and 3 IgG-negative sera. Overall, we found a very heterogenic immune response towards HEV proteomes, albeit with clear differences between seropositive and seronegative individuals. Furthermore, discriminatory immunogenic regions were identified in the N-terminal part of the capsid protein and at the C-terminal end of ORF3. These peptides can potentially serve as a starting point for the development of a peptide-based serological assay.

Conclusion

In conclusion, high density virus epitope microarrays are essential tools for the comprehensive identification of biomarkers for viral diseases such as HEV. The discovery of novel linear and conformational epitopes can lead to the development of innovative and multiplexed serological assays.

Technology



Benefits

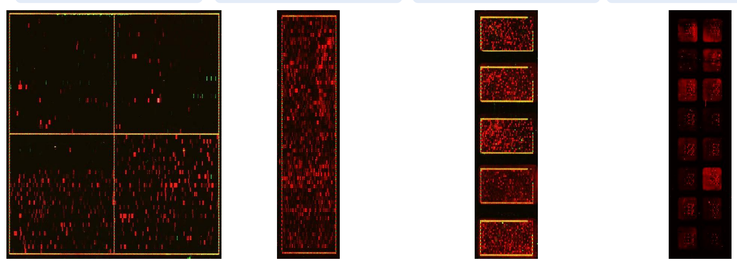
- very low material consumption
- high spot density / high content (800 peptides/cm²)
- digital printing flexibility, fast production times
- high peptide quality with routine double couplings

High-density PEPperCHIP® peptide microarrays are generated by digital laser printing on standard glass slides using a proprietary laser printer comprising 24 cartridges filled with individual amino acid toners. For array production, amino acid toners are simultaneously printed with high precision on their respective positions on the glass slides. Peptide synthesis is initiated by melting the toner particles to 90°C. Under these conditions, the amino acids are released and are available for coupling to the previous amino acids. The coupling cycle is completed by washing steps to remove excess building blocks and protecting groups. Finally, the array is ready for the next synthesis cycle with laser printing and coupling.

The benefits of this technique are an unique flexibility in terms of peptide content, a high spot density with up to 10.000 features per chip and low material consumption enabling the generation of customized peptide arrays at reasonable costs.

PEPperCHIP® Peptide Microarray Platform

Discovery Format | Standard Format | Mapping Format | Multiplexed Format

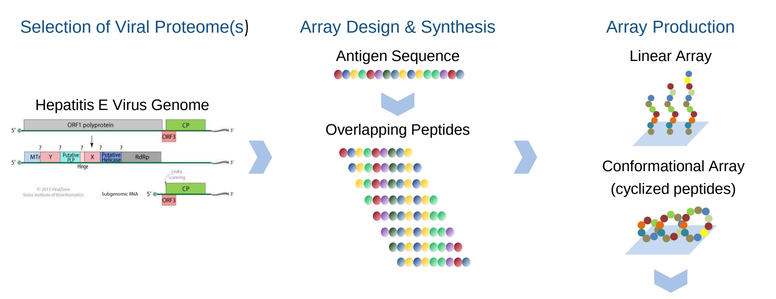


70,000 Peptides | ~11,000 Peptides | e.g. 5 x 1,200 Peptides | 16 x 200 Peptides

Antibody-peptide interactions are analyzed by immuno-type assays in a high-throughput fashion on peptide microarrays. Depending on the application, various microarray formats are available:

- **Discovery format**: approximately 70,000 individual peptides; suitable for screening of large, diverse epitope libraries or several full-length viral proteomes on a single chip; applied for epitope, biomarker and target binder discovery as well as antibody cross-reactivity analysis
- **Standard format**: covers approximately 10,000 individual peptides; routinely used for epitope mapping. Custom peptide microarrays or standard chips such as PEPperCHIP® Infectious Disease Epitope Microarray or the PEPperCHIP® Dengue Virus Type 1 Proteome Microarray among others
- **Mapping format:** several identical array copies on a single chip; ideal for parallel screening of multiple samples; used for epitope mapping of single protein antigens, detailed epitope characterization or biomarker validation
- **Multiplexed format:** up to 16 array copies on a single chip; ideal for assay development or hit validation studies with sample cohorts

Workflow Multiplexed Epitope Discovery



1: Chip with immobilized peptide antigens
2: Immunoassay
3: Fluorescence detection

- Hit confirmation on focused peptide arrays
- Further characterization, optimization & specificity testing

- Incubation with antiserum
- Staining of antibodies (here IgG) with secondaryAbs
- Fluorescence scan

Proteome-wide Linear and Conformational Epitope Mapping for Hepatitis E Virus

Hepatitis E Virus Epitope Microarray

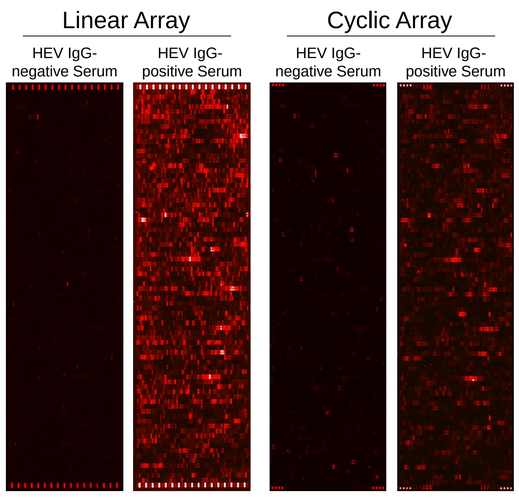
Array content:
- 5,426 overlapping peptides covering the whole proteomes of prototype strains of all human pathogenic HEV genotypes (genotype 1, 2, 3,4 and 7) plus controls; spots printed in duplicate

Linear Array:
- 15 amino acid peptides with 13 aa overlap

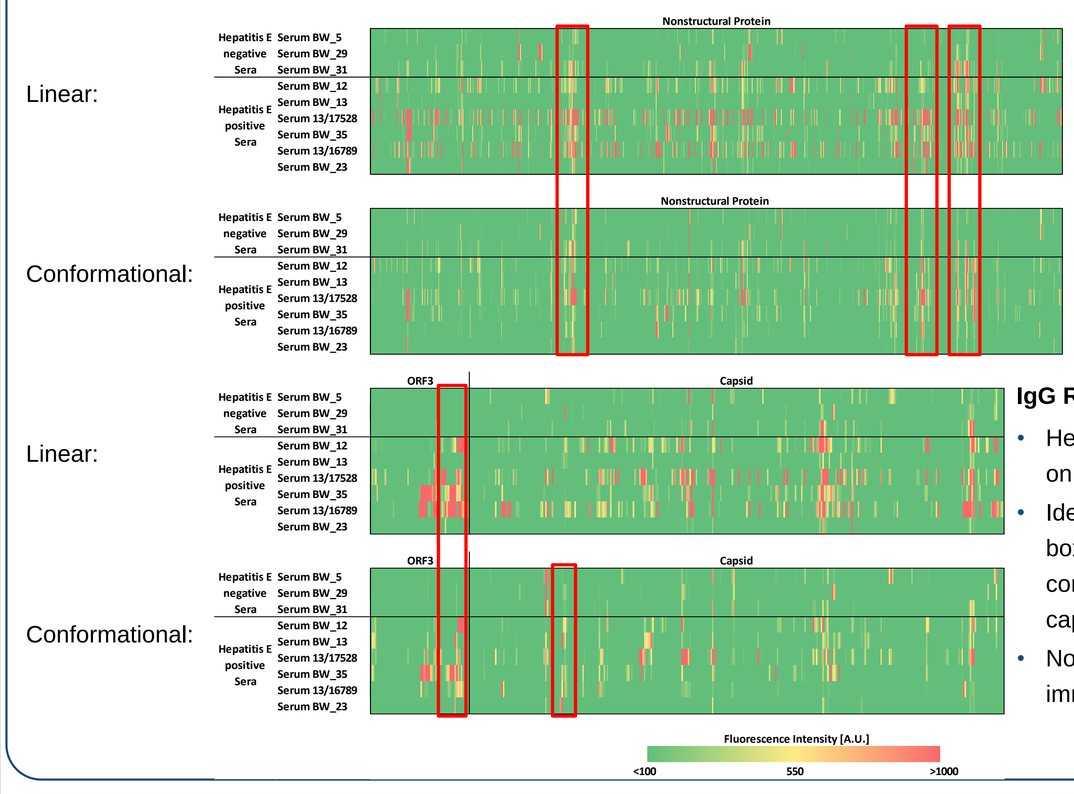
Conformational Array:
- 13 amino acid peptides with 11 aa overlap cyclized via thioether linkage

Samples:
- 3 anti-HEV IgG-negative and 6 anti-HEV IgG-positive sera determined by Euroimmun or Wantai HEV IgG ELISA

Assay:
- Serum dilution 1:200, detection with anti-human IgG DyLight680



Results – IgG Response against HEV Genotype 3



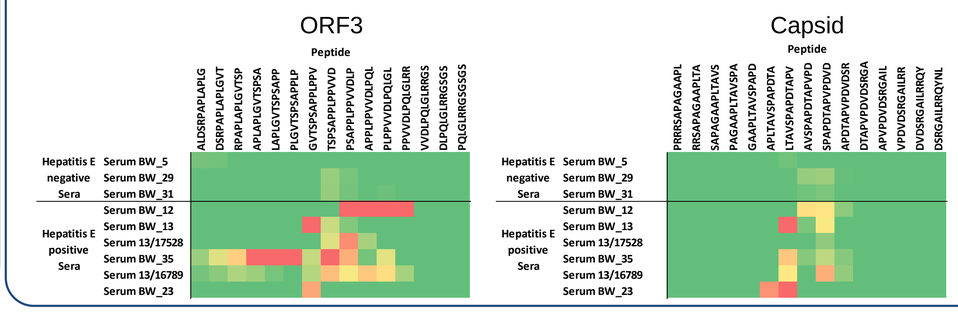
IgG Response against NSP-derived Peptides:

- Very heterogeneous immune response in individual patients
- Identification of highly immuno-reactive regions (see red boxes), but low discriminatory power
- Reactivity in HEV IgG-negative sera may result from old HEV infection with waning IgG titers that cannot be detected with current serological assays

IgG Response against ORF3 + Cap-derived Peptides:

- Heterogeneous immune response in individual patients on epitope level
- Identification of highly immunogenic region (see red boxes) at the C-terminal region of ORF3 (linear and conformational) and at the N-terminal region of the capsid protein (only conformational)
- No reactivity in HEV IgG-negative sera against immunogenic regions → high discriminatory power

In-depth Analysis of ORF3 and Capsid Immunogenic Regions



Results:

- All patients showed reactivity in immunogenic regions of ORF3 and capsid protein
- Considerable differences between individual patients on epitope level were observed
- Preferences for certain epitopes can probably be attributed to different HLA-types of patients
- These findings explain the poor performance of single peptide-based serological assays for HEV in the past

Summary

- Characterization of antibody responses towards Hepatitis E Virus on single epitope level
- High sensitivity at lowest sample consumption
- Identification of highly discriminatory immunogenic regions in ORF3 and capsid protein
- Combination of synthetic peptides is necessary for the development of specific and sensitive serological assays for Hepatitis E virus

Outlook

- Full substitution analysis of candidate peptides
- High throughput screening of validated peptides using large sample sets
- Assay development using validated synthetic peptides
- Formulation into standard assay formats such as ELISA