Introduction

All of us are infected by a number of viruses causing persistent infections and staying in our bodies for a long period of time, sometimes the whole life. While, in most healthy individuals not recognized, persistent viral infections can cause a number of serious health problems. EBV, for instance, the causative agent of infectious mononucleosis infects over 90% of the human population. The virus is thought to contribute to diseases such as different cancer types, multiple sclerosis and chronic fatigue syndrome. Probably, all of us feel sometimes infected. Additionally, persistent infections are a critical issue in organ and stem cell transplantation. The correlation of persistent infections with pathologies is an important issue; it is the gate to new diagnostics and therapies. However, passing the gate is still a demanding task. The detailed epitope mapping of humoral immune response in human serum samples allows a high resolution analysis of the antibody repertoire against EBV antigens. This information can then be correlated with clinical phenotypes. We developed a flexible peptide microarray platform which allows the presentation of up to 6900 peptides on a single microscope glass slide. Peptides are immobilized chemoselectively and directed onto the surface. A further highlight of the platform is the low volume of sample required; 1µl is enough. Serum incubations and data evaluation can be carried out using standard DNA-microarray equipment. We show examples where this approach allows to differentiate different donors for anti EBV humoral immune responses.

Library Design

- Overlapping peptide scans through major EBV antigens (BLRF2, BZLF1, EBNA1, EBNA3, EBNA4, EBNA6, LMP1, VP26) (1465 peptides)
- Follow-up library to include sequence variants and posttranslational modifications.

Array Production

Library generation

- SPOT-synthesis
- Synthesis of peptide with N-terminal reactivity tag
- Cleavage & reformatting
- Printing

QC-Scan & post-printing procedures

Chemoselective immobilization on microarray slides

Printing process

Fig. 1. Schematic representation of the array production process.

Fig. 2. Layout of peptide microarray (left) and images after printing (QC-scan, center) and after serum incubation (right). Three subarrays are used for improved data quality.

Fig. 3. Typical intra array reproducibility between the three subarrays.

Fig. 4. Reproducibility between different assays for one sample. The scatterplots show the mean signals per peptide in three independent experiments.

Seroscreening

- Screening of sera of healthy volunteers
- Evaluation of images using GenePix
- Processing of data and calculation for QC with R

Data Quality

- Individual signal patterns are observed for immunogenic proteins.
- Signals span along the peptide scan
- Development of a high content and high throughput screening platform for biomarker discovery
- Screening of low volume serum samples results in specific signal patterns
- Signal patterns for persistent infection can be compared to clinical data
- Library can be easily extended to sequence variants and posttranslational modifications.

Results

- Library Design
- Array Production
- Seroscreening
- Data Quality
- Results

References