Universal Mapping of Humoral Immune Response Using a Versatile High-Content and High-Density Peptide Microarray

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Introduction

Humoral immune responses are often the hallmark of efficient vaccines. The recent RV144 vaccine trial has turned attention to the stimulation of humoral immune response as a potential mode of action for HIV vaccines[1]. Therefore, detailed monitoring of antibody reactivities in patient specimens before and after vaccination is crucial. The determination of these reactivities on a sub-protein level provides information on the site of antigen/antibody interaction. In contrast to assays relying on whole antigens such as ELISA, peptide microarrays are efficient tools to deliver such information. Besides, complex peptide libraries can cover HIV sequence diversity, a special challenge provided by this virus.

Aim: Create peptide microarray presenting a versatile and comprehensive peptide library covering the immunogenic HIV proteome of multiple clades.

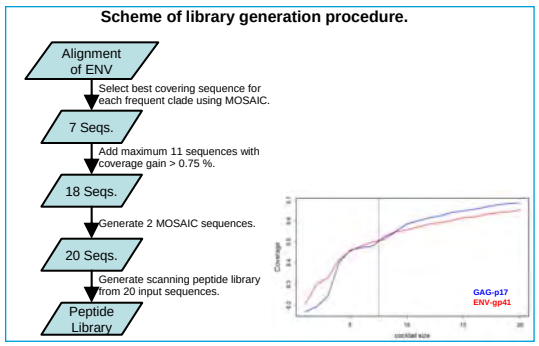

Fig. 1. Representation of coverage for cocktails of p17 (blue) and gp41 (red). The vertical line divides the cocktail into the 7 sequences for the frequent clades (left) and the added 13 sequences from the best covering sequences (right) as calculated against all HIV sequences irrespective of the clade.

Input Sequences

- Based on alignment of HIV transcripts from the LANL database version 2009[2].
- Full length of GAG (p17and p24), TAT, ENV (gp120 and gp41), and NEF.
- Immunogenic regions of GAG p2p7p1p6, POL, VIF, and REV as published by LANL[3].

Final Library

- Unique overlapping peptides.
- Clades A, B, C, D, G, CRF01_AE and CRF02_AG covered with at least one sequence.
- Inclusion of additional peptides to improve coverage of variable (V)- Loops
- The final library consists of 6565 peptides with an average coverage of > 50 %.

Coverage for ENV

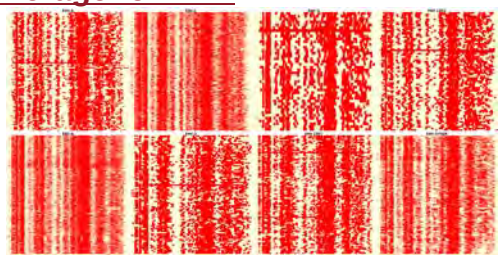

Fig. 2. Coverage of HIV1-peptide library for gp160 by clades (top row: A, C, G, CRF2; bottom row: B, D, CRF2, all other clades). x-axis: sequence of gp160, y-axis: all sequences for the respective clade from the alignment HIV1_ALL_2009_ENV_PRO.fasta are shown (total 2248).

Protein	number of source sequences	coverage (%)
ENV gp160	2248	60
GAG p17	3578	59
GAG p24	3578	86
NEF Nef	2606	55

Table 1. Average coverage of presented full length proteins.

Peptide Microarray Production

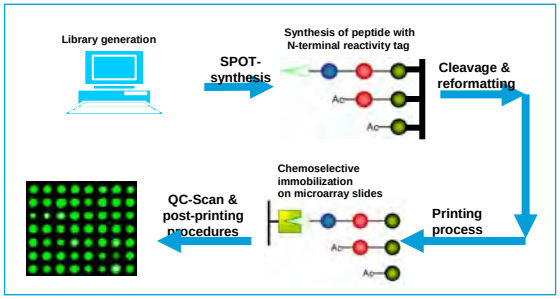

Fig. 3. Schematic representation of the array production process One synthesis batch allows the production of more than 1000 peptide microarrays.

Example Data

Fig. 4. Experimental data from patient serum. **Middle panel**: Alignment of presented peptides with underlying average signal intensity of overlapping peptides (white-weak:red-strong). The rectangle underlying the upper part of some letters represent the starting amino acid of a 15mere peptide. These rectangles are colored according to the signal intensity of the peptide. The boxplot right above the alignment represents the average signal intensity across all presented amino acids. **Upper panel**: Boxplot of signal intensities. **Lower Panel**: Consensus sequence of reactive peptides.

Summary

- **Development of a comprehensive HIV-1 peptide library with unprecedented coverage of clades.**
- **Flexible microarray platform allows easy adaption to answer specific questions.**
- **High resolution mapping of humoral immune response for different clades.**
- **Peptide microarrays were successfully used to study the humoral immune response in clinical vaccination trials.**

References

[1] Haynes et al.(2012) Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med.* **366**:1275-86.

[2] Los Alamos National Laboratory,http://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.htmlANL.

[3] "HIV Molecular Immunology: Maps of Ab Epitope Locations Plotted by Protein" version March 5, 2010 from Theoretical Biology & Biophysics, Los Alamos National Laboratory was used (http://www.hiv.lanl.gov/content/immunology/maps/ab/ab.pdf).

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