

The Challenge of Complexity: Peptide Tools for the Development of Immunotherapies

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Introduction

Immunotherapy is gaining attention as promising approach to fight cancer as well as infectious diseases.¹ A major challenge in immunotherapy is the selection of optimal antigen sequences to derive efficient therapeutic agents. Among others, this task is hampered by sequence diversity in the target organisms caused by isoforms, splice variants, polymorphisms, mutations, and PTMs. An example is the Human Immunodeficiency Virus (HIV), for which thousands of different sequences are known just for the envelope protein alone (Figure 1).



Figure 1: Sequence variability in the HIV Nef protein (aa20-67 in reference strain HXB2). One representative sequence per frequent clade is shown.

Methods

We address the challenge of sequence diversity by a peptide library based workflow that combines:

Improved bioinformatic algorithms:

New algorithms for library design were developed. These are based on the scoring of all possible peptides according to their frequency of occurrence across all sequences to provide the most homogenous overall coverage.² The result of the so-called Ultra Concept is illustrated in Figure 2 for the HIV Nef protein. The majority of the 3903 known sequences is covered by only 150 peptides (for antigen specific T-cell stimulation with peptide pools) or 667 peptides (for humoral immune monitoring with peptide microarrays).

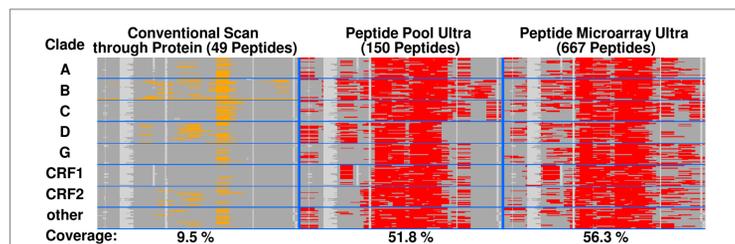
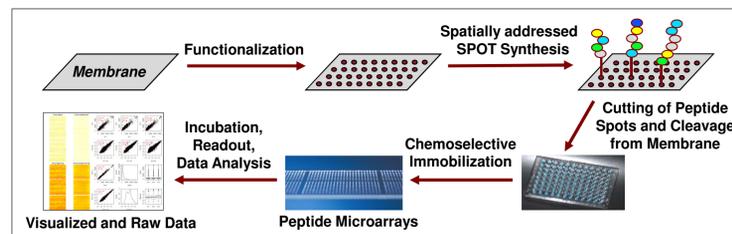


Figure 2: Sequence coverage by different HIV Nef libraries. Red/orange: Sequence parts which are covered by the respective peptide library.

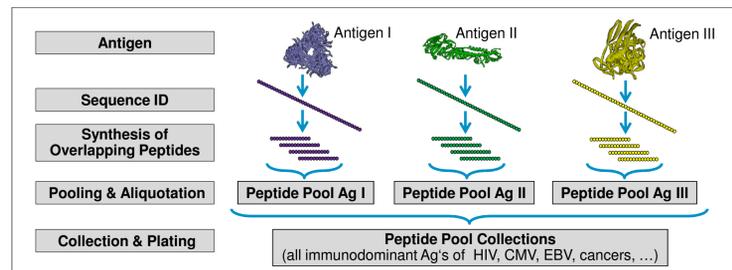
High throughput peptide synthesis, peptide presentation and synergistic assay formats:

For B-cell epitope discovery and humoral immune monitoring high density peptide microarrays represent an efficient technology that accommodates vast numbers of sequence variants and PTMs. Peptides are synthesized by SPOT synthesis³ and re-immobilized on microarrays⁴ in a clean-room environment (Scheme 2). Readout is usually performed by fluorescently labeled secondary antibodies.



Scheme 1: Preparation of peptide microarrays to study humoral immunity.

For T-cell epitope discovery and cellular immune monitoring peptides selected by the Ultra Concept can be synthesized and presented as individual peptides, matrix pools or antigen spanning pools for application in T-cell assays such as Elispot (Scheme 2).



Scheme 2: Preparation of peptide pools to study cellular immunity.

Application Examples

Humoral immune response:

To provide guidance for vaccine development, plasma samples from several HIV/SIV vaccination studies were examined with peptide microarrays.^{2,5} The analysis of serum samples from the first successful HIV vaccination trial (RV144 trial) with peptide microarrays showed that plasma levels of IgG directed towards the V2 loop of gp120 correlated with a reduced risk of infection.^{5a} In a follow-up study, an Ad26 vector-based vaccine stimulated a dose dependent response against the V2 loop (Figure 3).^{5b}

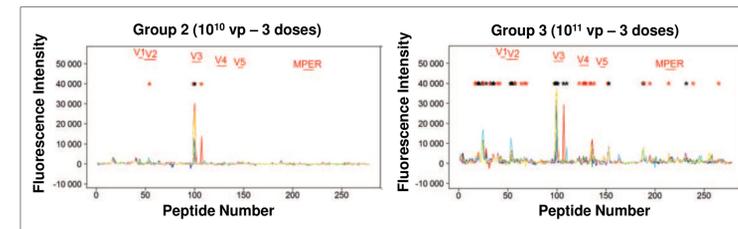


Figure 3: HIV Env-specific antibody responses using different dosing regimens (IPCAVD 001 trial).

Cellular immune response:

To increase stimulating efficiency for antigen specific T-cell responses (HLA independence, reduction of assay numbers, sample volume requirements), antigen-spanning overlapping peptide pools were developed. Examples include pools for the *ex vivo* generation of T cells for HIV⁶ (Figure 4) and broad-spectrum antiviral (AdV, EBV, CMV, BKV, HHV6)⁷ treatment, where a 94% virological and clinical response rate was achieved.

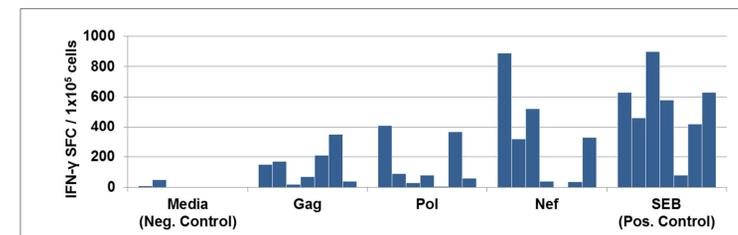


Figure 4: HIV-specific T cell responses after *ex vivo* expansion of T cells from seven HIV+ donors with Ultra peptide libraries.⁶

Summary

To address the challenge of sequence diversity in immunotherapy, a peptide based workflow was established that combines bioinformatic algorithms, high throughput peptide synthesis, innovative peptide presentation approaches and synergistic assay formats.

References

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