



Antibody signatures defined by high-content peptide microarray analysis

Circulating antibodies are highly selective binding reagents directed to a vast repertoire of antigens. Candidate antigens displayed as overlapping peptides on PepStar™ peptide microarrays can be used to screen for recognition by serum antibodies from clinically well defined patient populations. The methodology is robust and enables unbiased visualization of antigen-specific B-cell responses. Additionally, autoantibody signatures of diagnostic value could be detected using microarrays displaying thousands of human peptides.

Antigen arrays have been used to gauge humoral immune responses. Using PepStar high-content peptide microarrays displaying overlapping peptides in the contexts of infectious and autoimmune diseases, allergy and cancer, we have improved the resolution of antigen arrays down to the epitope level.

A detailed knowledge of the target epitopes from pathogens recognized by B cells from either vulnerable or protected individuals enables rational vaccine design and facilitates development of novel diagnostic tools. Currently, our knowledge of epitope-specific recognition patterns is limited to small regions of the proteomes of pathogens with large genomes. Recently, PepStar high-content peptide microarrays were used to profile antibody response to *Mycobacterium tuberculosis* epitopes^{1,2} and allowed an unbiased and global view of the immune response, without the need to preselect target proteins. In that work, PepStar microarrays displaying 7,446 unique *M. tuberculosis* peptides together with 153 negative controls (printing buffer without peptides deposited), 24 positive controls (human immunoglobulins immobilized) and 96 fluorescent landmarks had been treated with sera from 34 individuals with active pulmonary tuberculosis and 35 healthy individuals to extract differences and antibody signatures predictive of tuberculosis. Linear 15-residue peptides derived from *M. tuberculosis* proteins and overlapping by 3 amino acids had been printed on functionalized glass slides, attached through a linker on the N terminus of the peptides. Resulting PepStar peptide microarrays can be stored at 4 °C for up to 18 months. Sera from subjects with active pulmonary tuberculosis were diluted 1:100 using a buffer consisting of PBS, 3% FCS and 0.5% Tween and placed onto the microarray surface (300 µl total volume, only 3 µl of serum per slide), incubated at 4 °C for 16 h, washed and dried, and

IgA and IgG peptide complexes were visualized using fluorescently labeled secondary anti-human IgA or IgG reagents.

In this analysis, three distinct patterns of antibody reactivity were identified: (i) peptides that were differentially recognized between tuberculosis-positive and healthy individuals, (ii) peptides that were exclusively recognized in all individuals with tuberculosis but not in any of the healthy ones, and (iii) peptides that were exclusively recognized in healthy but not tuberculosis-positive individuals. Antibody signatures in serum from tuberculosis-positive subjects from Armenia versus ones recruited in Sweden showed that IgG-defined tuberculosis epitopes are very similar in individuals with different genetic backgrounds. Notably, differentiation between tuberculosis-positive and healthy individuals does not cluster into specific recognition of distinct *M. tuberculosis* proteins, but into specific peptide epitope 'hotspots' at different locations within the same protein.

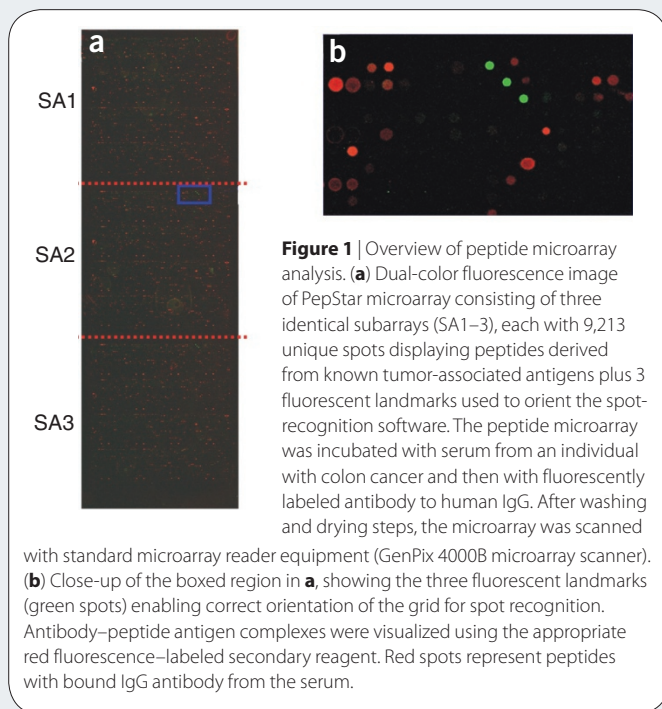
The use of PepStar peptide microarrays is not limited to the detection of antibody signatures directed against foreign antigens in infectious diseases^{1–3} and allergies^{4–6}. Peptide microarrays displaying citrullinated peptide derivatives derived from human proteins enabled detection of induction of antigen-specific tolerance in multiple sclerosis after immunization with DNA encoding myelin basic protein in a randomized, placebo-controlled, phase 1/2a clinical trial⁷. The research used cerebrospinal fluid to probe autoantibody binding to PepStar microarray-bound peptides and demonstrated that the DNA vaccine produced cross-tolerance to other antigenic components, such as proteolipid protein. Moreover, PepStar microarrays displaying overlapping peptides derived from autoantigens are a useful tool to detect diagnostic autoantibody signatures in autoimmune diseases such as systemic lupus erythematosus⁸.

Finally, peptide microarrays can be used for the reliable detection of autoantibodies with low serum titers, such as antibodies to tumor-associated antigens in individuals with cancer. We obtained a fluorescence image of a peptide microarray treated with serum from an

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APPLICATION NOTES



individual with colon cancer (**Fig. 1**). (Sera were obtained according to the rules and with the approval of the local ethics committees (“Gewebesammlung für molekularbiologische Untersuchungen bösartiger und gutartiger Erkrankungen des Gastrointestinaltraktes und endokriner Organe”; II HV 43/2004) and kindly supplied by M. Linnebacher, Section Molecular Oncology and Immune Therapy; University of Rostock, Germany.) The resulting antibody signatures from individuals with colon cancer together with the signatures from healthy individuals enable identification of unique peptide sequences for the development of new diagnostic or prognostic peptide chips.

Conclusion

PepStar high-content peptide microarrays are a robust platform for screening entire proteomes for antibody recognition. This has the potential to define biologically relevant targets in the context of rational vaccine design and development of novel diagnostics.

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