

Humoral 'reactome' profiles using peptide microarray chips

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In their recent article, Prechl and coworkers discussed the use of protein antigen microarrays as a viable platform to gauge the humoral 'immunome' or 'reactome' against target proteins [1]; an approach which has successfully been used to gauge autoimmune responses [2]. Peptide microarrays might exhibit a certain advantage over protein

antigen microarrays in that they are able to identify the immunoglobulin recognition hotspots on the nominal target protein. Our knowledge of epitope-specific recognition patterns is limited to small regions of the proteome [3] of pathogens with large genomes. Peptide microarrays have recently been used to profile antibody response to simian

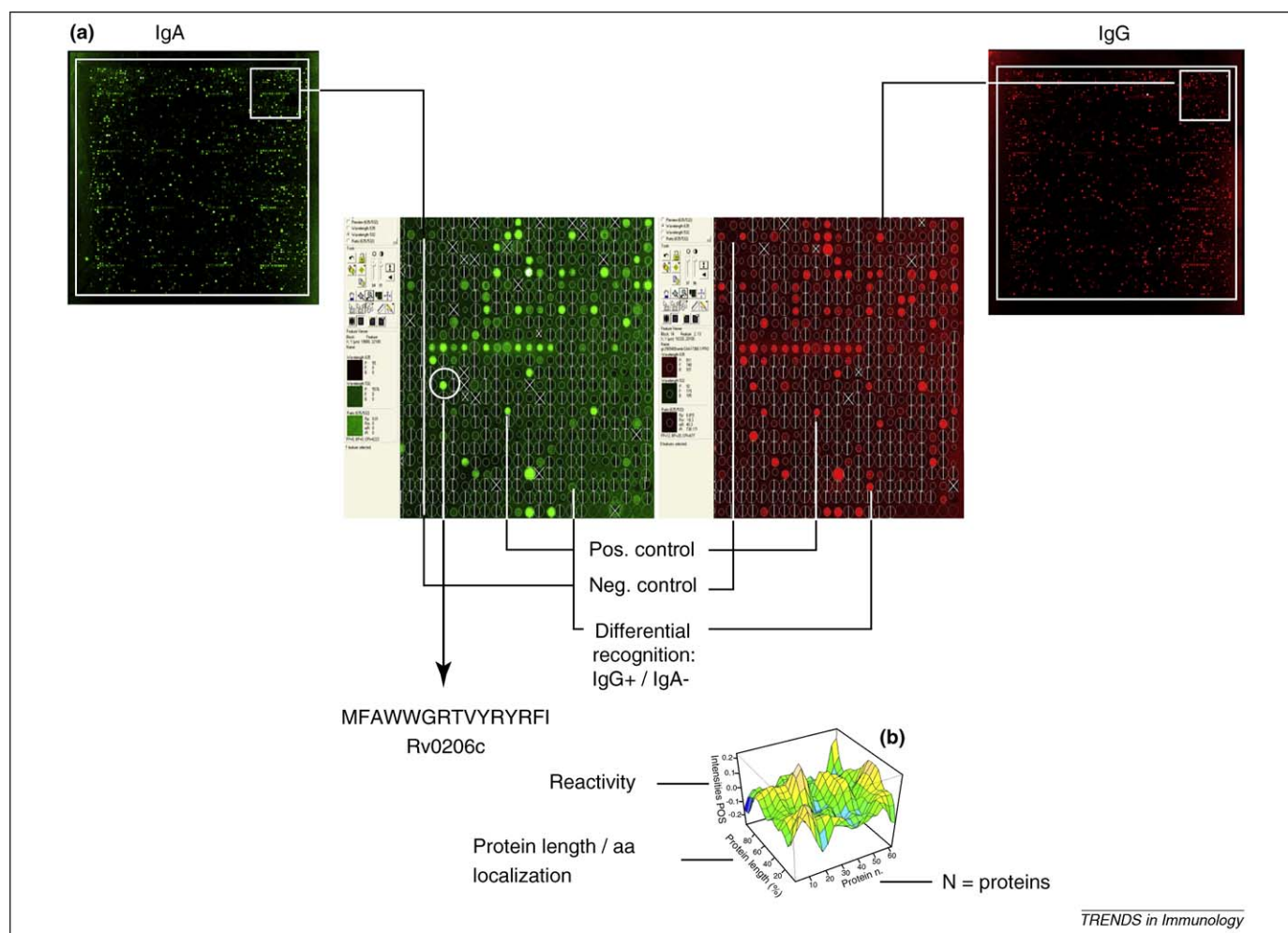


Figure 1. Overview of peptide microarray chip analysis. The analysis platform (left for IgA, right for IgG) consists of three identical sub-arrays, each with a unique set of 9216 spots from *M. tuberculosis* plus 240 positive control peptides for the secondary reagent and 141 negative control spots. **a.** Magnification shows the Gal file (mask) that allows the identification of the amino acid sequence of each peptide printed in a designated location. Serum is tested for IgG or IgA binding to peptides and the slide is scanned. Positive controls detect the function of the secondary reagent; empty spots are devoid of peptides; serum antibody-peptide antigen complexes are visualized using the appropriate secondary reagent. The plot shows a representative positive result with a target epitope amino acid sequence identified. Some epitopes are uniquely recognized by IgG or IgA. **b.** 3D model of serum reactivity directed a comprehensive set of Mtb targets. Sixty-three proteins were tested and the amino acids were localized within the respective protein. Antibody binding was measured as epitope-specific fluorescence intensity. Note the hotspots of recognition of individual proteins and 'ignorance' of certain target structures displayed by the antibody-recognition matrix. The data set has been published earlier and refers to differential 'recognition hotspots' in *M. tuberculosis* proteins [5].

immunodeficiency virus (SIV) [4] and *Mycobacterium tuberculosis* epitopes [5], and have allowed an unbiased, global view of the immune response, without preselecting target proteins. In this technique, linear peptides are produced by SPOT synthesis and then printed on epoxy functionalized glass slides via a linker on the N-terminal side of the peptide [5,6]. Of note, it was not protein-specific immunoglobulin responses, but rather peptide-specific responses that helped to discern between serum reactivity in different SIV vaccine regimens and SIV wild-type infection [4]. Furthermore, a comparison between serum reactivity in patients with pulmonary tuberculosis and healthy control individuals has shown that significant differences in serum reactivity are not related to *M. tuberculosis* protein target recognition, but to different hotspots within the target proteins [5]. At least two outcomes are possible in this context: differential recognition of epitopes, that is, peptides are recognized from immunoglobulin present in the test group and in the control group, but with different strength (strong recognition in one group *vs.* weak recognition in the other group), as well as 'exclusive recognition' of peptide targets that are recognized in serum from a test group but never in a control group (or vice versa). A disadvantage of peptide microarrays is, at least in the current format, that only linear epitopes are displayed and the biological consequence of antibody binding to these epitopes remains to be shown. More recently, peptide antigen-specific humoral immune responses have been shown to be instrumental for characterization of natural antibodies directed against amyloidogenic peptides in patients with Alzheimer's disease [7]. This underlines an important point. Physicians and researchers have demonstrated the increase in humoral reactivity, but the absence of immunoglobulin reactivity is hard to demonstrate and standardize. However, it is biologically relevant: immunoglobulin reactivity in serum shapes a recognition matrix of 'self' and 'non-self' antigens, which has been termed the 'immunological homunculus' [8]. The absence of certain epitope-specific reactivity in the case of antibodies directed against neuronal structures has been associated with increased risk of Alzheimer's disease [7]. The presence of certain (natural) antibodies might be associated with a 'nourishing function', for example, by inducing specific cellular signaling events after engagement of cell surface

receptors (discussed in ref. [9]). Thus, a detailed description of epitope-specific recognition to visualize the global increase and decrease or loss of immunoglobulin reactivity might be advantageous to understand the pathological mechanisms of certain diseases, or to describe the effects of aging on the humoral immune system [10] using a comprehensive peptide matrix as a readout model. Figure 1 shows an example of a simultaneous screening of 9216 unique peptides that represent 324 proteins from *M. tuberculosis* tested for IgA and IgG reactivity, and a 3D model of immunoglobulin reactivity [5]. 'High-content' peptide microarrays, guided by quality data extraction, represent robust platforms to screen entire proteomes for antibody recognition. This has the potential to define the presence and absence of immunoglobulin reactivity towards biologically relevant (peptide) targets or epitope patterns in a more global and systemic view of the immunoglobulin 'reactome' in health and disease.

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