

Systematic Antigen-Based Plasma Profiling in Multiple Sclerosis

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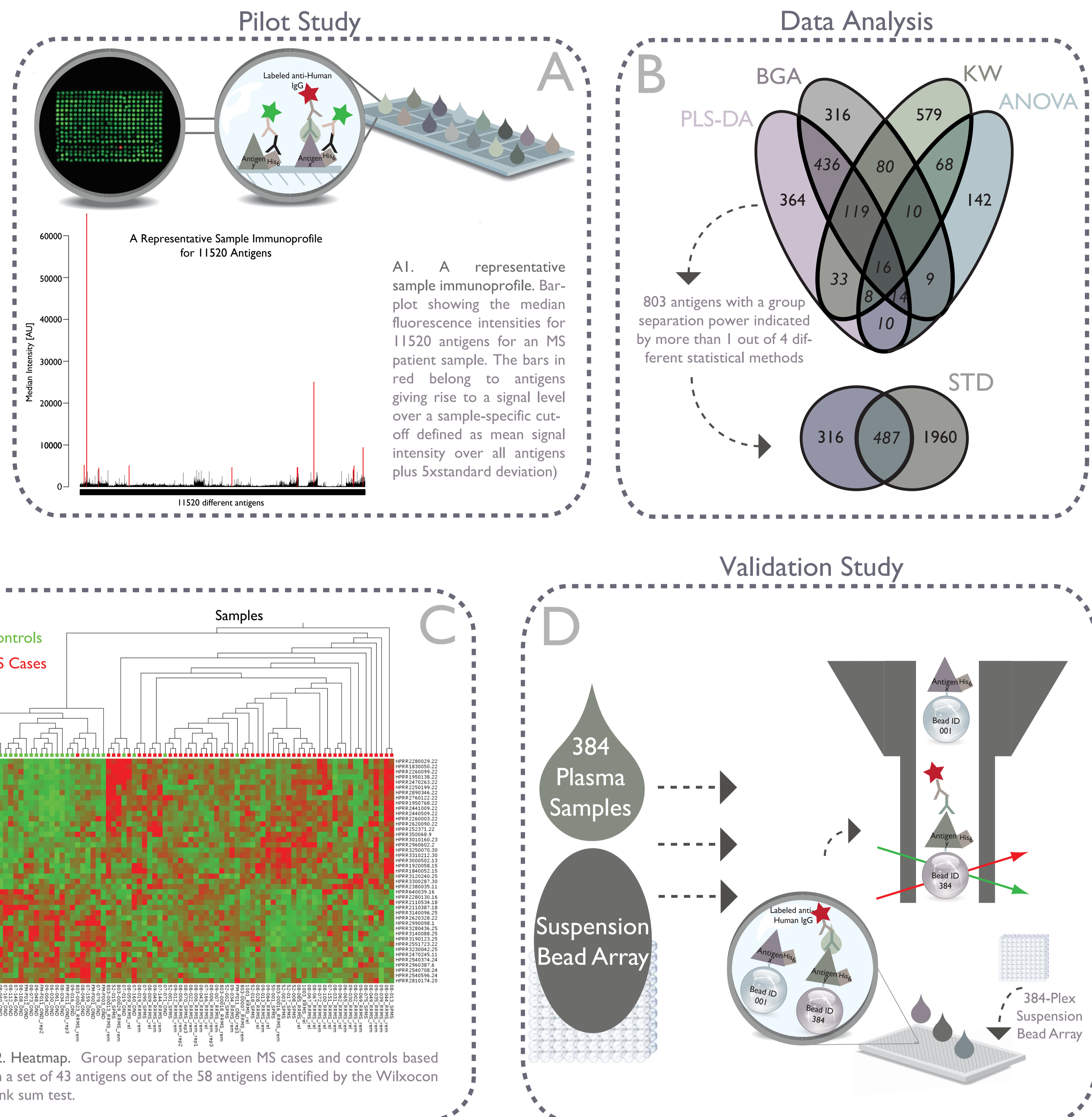
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Introduction

Systematic and antigen-based profiling of the autoantibody repertoire in disease-related plasma provides a powerful strategy for the investigation of new, self-reactive immune responses in diseases such as multiple sclerosis (MS).

The presented study aims at such a systematic screening to unveil autoimmunogenic features in MS by using a large collection of human antigens generated within the Human Protein Atlas Project. These antigens are used to produce antigen microarrays, which for this project has served as a discovery platform to identify potential MS-related autoimmunity targets in plasma.



B1. Volcano plot showing the comparison between MS cases and controls. Group comparison was performed using Wilcoxon rank sum test and obtained *p*-values were plotted against the fold changes. 58 antigens (in green) showed a significance levels below 0.01, indicated by the green line.

B2. Heatmap. Group separation between MS cases and controls based on a set of 43 antigens out of the 58 antigens identified by the Wilcoxon rank sum test.

Methods and Results

The antigen microarrays used in the pilot study contain 14 replicated sub-arrays, each with 384 different antigens. 90 individual samples (including 61 MS cases and 29 controls) are employed onto 30 different batches of these arrays, allowing to obtain immunoprofiles for 11520 different antigens. The assay conditions are optimized to reduce background binding and the binding events are detected by an anti-human IgG fluorescence-labeled antibody (Figure A).

Data is analyzed by applying 4 different statistical methods and 803 antigens are identified with a group separating power

indicated by more than 1 out of the 4 methods (Figure B). The volcano plot and heatmap in Figures C1-C2 represent an example group comparison between MS cases and controls based on Wilcoxon rank sum test. A further sample-specific filter as exemplified in Figure A1 is applied, resulting in 487 antigens eligible for a further validation (Figure B). A selection of 384 antigens out of this set are coupled with 384 different color-coded beads, forming a suspension bead array (Figure D). Using this alternative technical setup, an extended MS cohort is studied to verify the findings of the pilot study.

Conclusion

The presented study aims at the identification of MS-specific immunoprofiles in plasma using a systematic, antigen-based plasma profiling approach based on planar antigen microarrays and at the simultaneous validation of these immunoprofiles in an extended sample cohort using suspension bead arrays as an independent technical platform. This multi-parametric characterization of immunoprofiles will provide an avenue towards new autoimmune patterns that can be linked to MS.

References

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