Evaluating the Shared Epitope Phenomenon Using Expanded Single Antigen Bead Panels

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Background

Single antigen bead (SAB) technology revolutionized HLA antibody testing, enabling simultaneous interrogation of almost 100 different antigen targets. However, the homologous nature of HLA proteins leads to a significant limitation of this multiplex assay, as antibodies recognizing epitopes shared by multiple antigens can result in a dilutional effect, and thus an artificially lower median fluorescence intensity (MFI) per bead. This study investigated novel, expanded SAB panels for Class I and II with 147 and 140 HLA antigen-coated beads, respectively, and compared to standard 96-bead panels, to determine if the shared epitope phenomenon is affected by an increase in multiplexing and the potential binding targets.

Methods

We tested serum samples from 47 patients with both the LIFECODES® Single Antigen (LSATM) and expanded (LSATM NEXA) reagents for Class I and II. The LSATM Class I and II panels consist of 96 HLA-bearing microbeads, while the Expanded NEXA panels include an additional 51 and 44 beads, respectively. The background-adjusted MFI for the 96 beads that are shared by the panels were compared for each sample, and this was also compared to with the percentage increase in putative targets on the panel for each patient's antibody profile, based on expected reactivity to certain Class I CREGs or Class II shared epitopes.

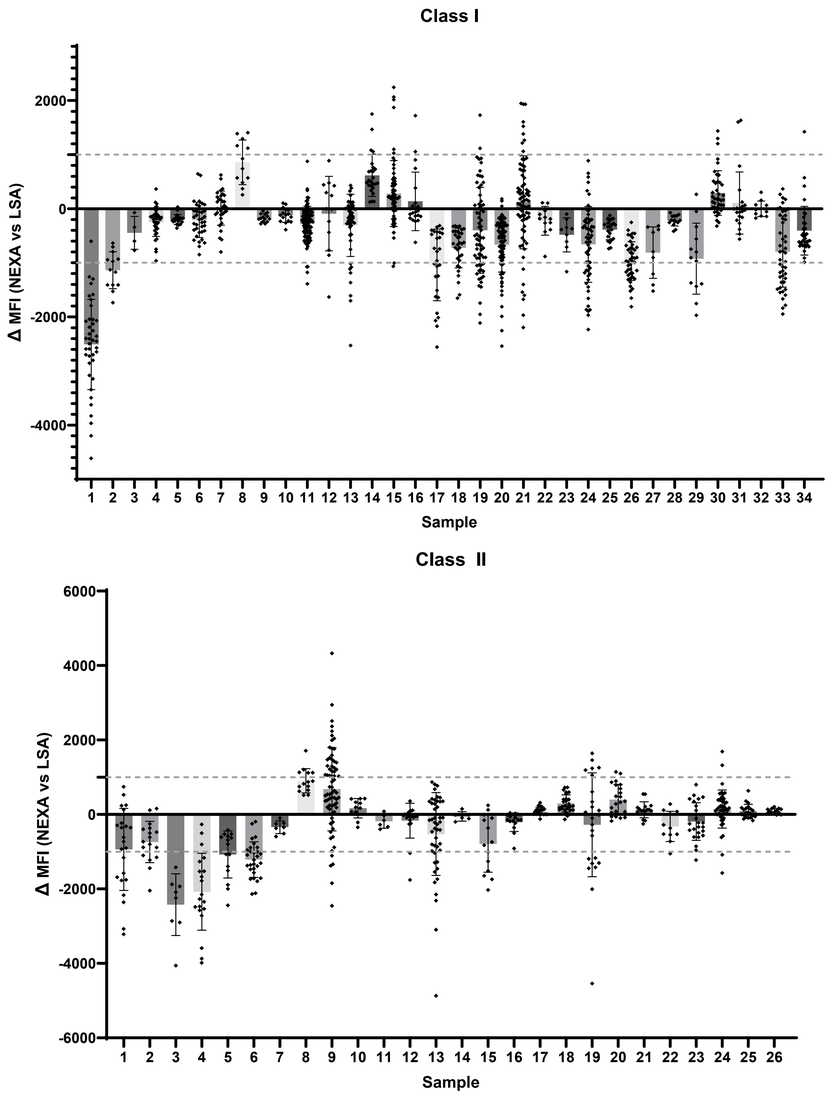

Figure 2. Change in MFI for 96 shared beads on NEXA panel compared to LSA panel for each sample with positive specificities detected for Class I (A) and Class II (B).

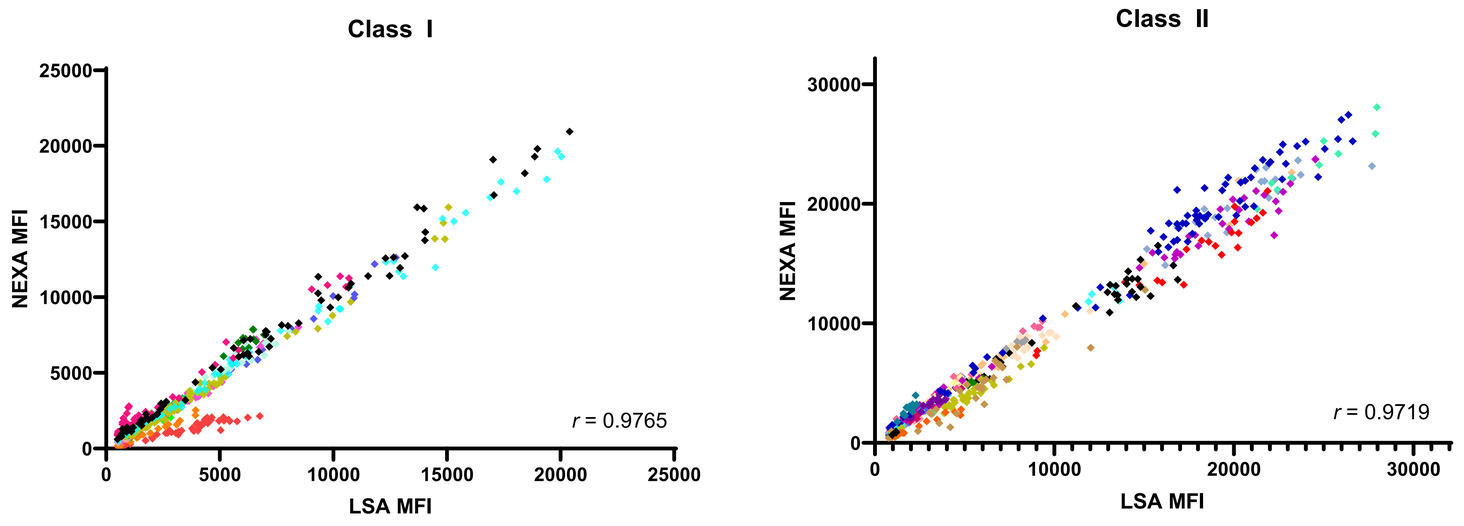

Figure 1. MFI values for the 96 shared beads on the LSA and NEXA Class I (A) and Class II (B) panels for all samples tested with Pearson r correlation.

Results

Of the 47 samples tested, 34 and 26 were positive for antibodies targeting Class I or Class II shared epitopes, respectively. While a majority of the samples demonstrated comparable MFI values across both assays, and overall correlation of MFI was strong when combining all samples (Figure 1), there were 14/34 Class I and 9/26 Class II samples that demonstrated a significant reduction in MFI across the common beads on the two panels, with an average delta MFI per bead of -866 for Class I and -994 for Class II in this group (Figure 2). When comparing the change in MFI against the specific shared epitope targeted, median MFI values observed, or percentage increase in the number of bead targets, there did not appear to be any significant association with any of these sample characteristics and a worsening of the shared epitope phenomenon (Figure 3).

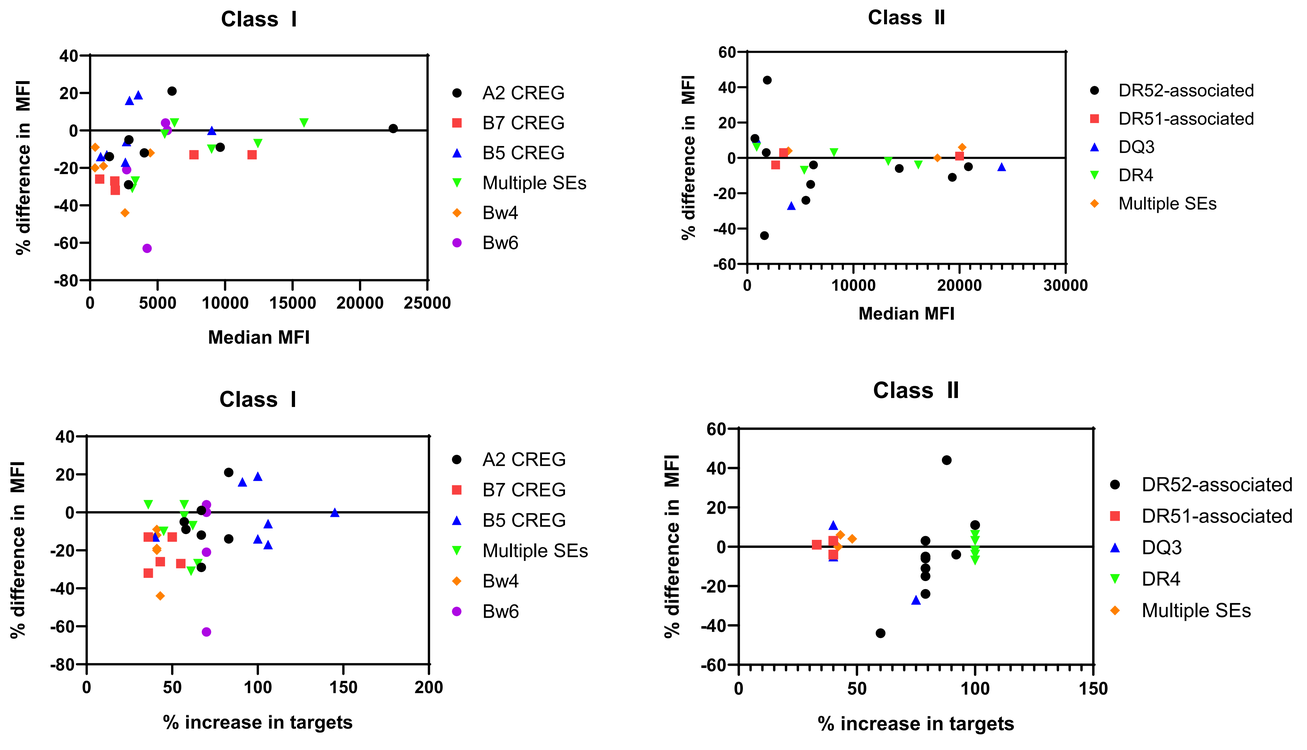

Figure 3. Correlation of Median MFI value (A and B) and the percent increase in binding targets (e.g. 4 beads on LSA → 8 beads on NEXA = 100% increase) (C and D) with % difference in MFI for the relevant beads based on sample-specific shared epitope.

Conclusions

In conclusion, it appears that while some samples demonstrate lower MFI valuesper target bead following an increase in binding sites, others are unaffected. Further work is needed to understand the mechanisms underlying these differences, and these reagents may prove useful in discriminating between samples that may be subject to shared epitope-related underestimation of antibody strength and those that are not. This would be invaluable in our understanding of this assay limitation and interpretation of virtual antibody assessments in support of solid organ transplant.

Acknowledgements

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