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Human Leukocyte Antigen Epitope Definition Using Site-directed Mutagenesis

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Recently, there have been great advances in our understanding of the nature of HLA antibody binding at the epitope level. There are various tools available to facilitate this such as HLA Matchmaker, HLA-EMMA, and electrostatic mismatch scoring. There are many examples where multiple amino acid residues may be responsible for antibody binding, so it is not possible to isolate the exact residue implicated due to sharing across multiple HLA antigens. An example of this is the reaction pattern positive on the single antigen bead assay only for HLA-A1 and A36 which could be due to recognition of any one of three shared polymorphic residues(figure 1). To investigate this we performed site directed mutagenesis to isolate these residues and assess the reactivity of human monoclonal antibodies (mAb) against these HLA variants using flow cytometry. With this approach we were able to identify that the binding to HLA-A1 and A36 of the human mAb VDK1D12 was specific for lysine at position 44(figure 1). Additionally, using this approach we were able to define the critical binding residues for a number of HLA-DQ specific mAbs. We were able to demonstrate abrogation of binding following mutation of critical residues but importantly, we were also able to demonstrate that binding could be induced by the substitution of a critical amino acid residue to a previously non-reactive HLA allele. Site-directed mutagenesis of HLA molecules offers a reliable method for defining the amino acid residues crucial for HLA antibody recognition. This innovative technique offers a unique opportunity for us to further our understanding of HLA immunogenicity and allow for the in vitro validation of many epitopes currently listed as not antibody verified.