HLA-DP Antibodies Targeting Combined DPB1/DPA1 Epitopes

Danny Youngs, Idoia Gimferrer, Elaine Chou-Wu
Bloodworks Northwest, Seattle, WA

Introduction

From previous studies by our lab and others, antibodies to HLA-DP appear to target a very limited set of epitopes. Almost all DP antibodies appear to target one of the following eplets. DPB1: 84DEAV, 85GPM, 57D (*55DED*), rp58EE (*55DEE*), rp58E (*55DED/DEE*), 55A (*55AAE*), 55EAE, 56A (*55AAE/EAE*), 69R, 96K, 96R; DPA1: 50Q, 50R.

In this study we report cases of DP antibody reactivity that can best be explained as antibodies simultaneously targeting epitopes in both the DP-alpha and DP-beta chains. These are not combinations of antibodies (separate antibodies to DP-alpha and DP-beta in one serum) but single antibodies targeting combinations of DP-alpha and DP-beta epitopes that are brought in close proximity in the folded DP molecule. These types of antibodies are hereafter in this poster referred to as DPB1xDPA1 antibodies.

Methods and Materials

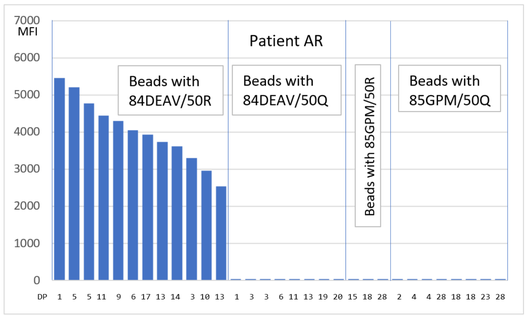
Approximately 85,500 samples from 13,500 transplant patients were tested for HLA antibodies using LABScreen Single Antigen (LSA) (One Lambda), with data collected in the HLAFusion database (One Lambda). Mean Fluorescence Intensity (MFI) data in HLAFusion was queried for patterns of reactivity that correspond to a combination of a DP-beta epitope in conjunction with a DP-alpha epitope. Every possible combination of DP-beta and DP-alpha epitopes was searched.

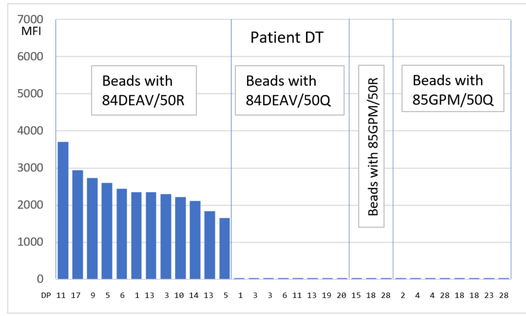
To confirm that DPB1xDPA1 bead reactivity patterns were not artifacts of the LSA assay, flow cytometry crossmatches were performed between DPB1xDPA1 sera and B-cells homozygous for each of the various combinations of 84DEAV and 85GPM with 50Q and 50R.

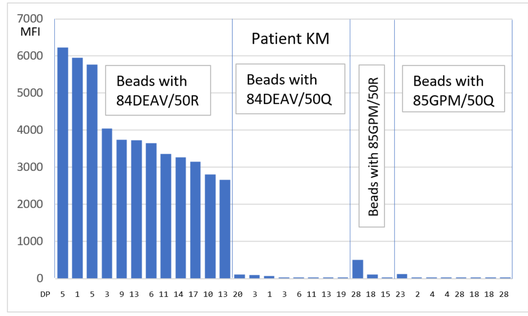
Results

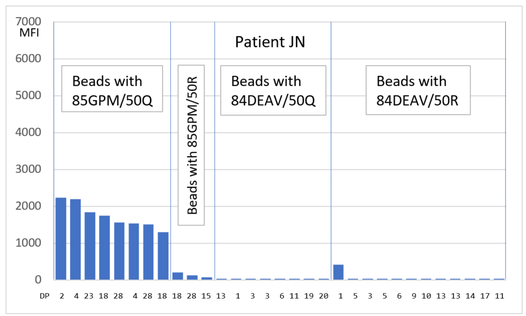
A total of seven patients were identified who had LSA patterns consistent with DPB1xDPA1 antibodies: 4 patients had antibodies to 84DEAVx50R (including patient AR, whose donor interestingly does not have 50R), 2 patients with antibodies to 85GPMx50Q (including patient JN, who is 50Q herself), and 1 patient with antibodies to 85GPMx50R (even though patient ST is 50R herself). The MFIs of the DP beads in the LSA panel are graphed for each patient (above, right).

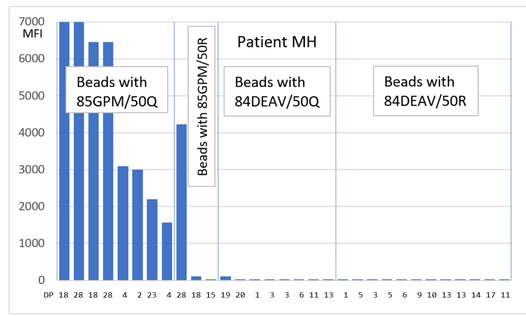
The table displays the DP types of these patients, the DP types of their organ donors (most of these patients exhibited their DPB1xDPA1 antibodies in post-transplant samples), and the B-cell XM results with target cells bearing different combinations of 84DEAV and 85GPM with 50Q and 50R. Because some sera had extensive antibodies to other HLA, informative crossmatches could not be performed with those sera or certain serum/cell combinations; in those cases the crossmatch results are left blank.

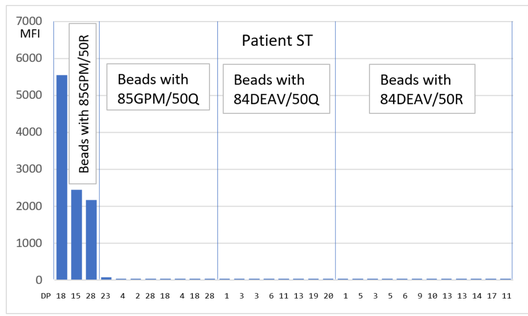




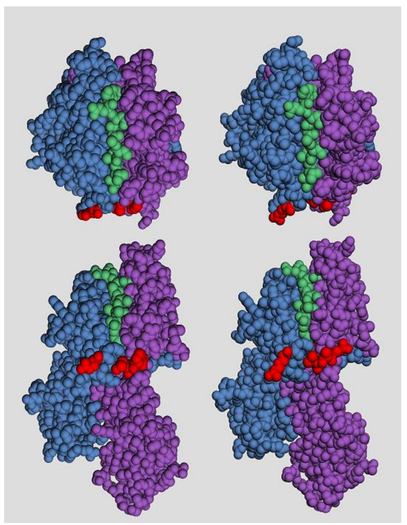








Patient	Patient DP Type	Graft DP Type	Antibody Specificity	B-cell XM with 84DEAV/50R Cell 1	Cell 2	B-cell XM with 84DEAV/50Q Cell 3	Cell 4	B-cell XM with 85GPM/50Q Cell 5		B-cell XM with 85GPM/50R Cell 6	Cell 7
AR	DPB1*04:01 DPA1*01:03	DPB1*02:01, 03:01 DPA1*01:03	84DEAVx50R	neg	POS	neg	neg	neg		neg	neg
DT	DPB1*04:01, 04:02 DPA1*01:03	No Transplant	84DEAVx50R	POS	POS	POS	POS	neg		neg	
KM	DPB1*04:01, 15:01 DPA1*01:03, 01:04	DPB1*01:01, 04:01 DPA1*01:03, 02:01	84DEAVx50R	POS	neg	neg	neg	neg		neg	neg
JB	DPB1*02:01, 04:01 DPA1*01:03	DPB1*03:01, 17:01 DPA1*01:03, 02:01	84DEAVx50R								
JN	DPB1*30:01, 104:01 DPA1*01:03	No Transplant	85GPMx50Q	neg	neg	neg	neg	POS		neg	
MH	DPB1*17:01, 63:01 DPA1*02:01	DPB1*04:02, 14:01 DPA1*01:03, 02:01	85GPMx50Q	neg	neg	neg	POS	POS		neg	
ST	DPB1*01:01, 17:01 DPA1*02:01	DPB1*02:01, 11:01 DPA1*01:03, 02:01	85GPMx50R								



Models of DPB1*04:01/DPA1*01:03 (left figure) and DPB1*01:01/ DPA1*02:01 (right figure)

Blue = DP-alpha chain
Purple = DP-beta chain
Green = peptide
Red = amino acids at position 50 (DP-alpha) and positions 84-87 (DP-beta) that are the likely targets of DPB1xDPA1 antibodies.

DP model images from http://www.phla3d.com.br/

Discussion

Although these patients' LSA patterns appear to clearly target a conjunction of DP-alpha and DP-beta epitopes, the crossmatches are not nearly as clear. This may be due to the generally low MFIs of these antibodies as well as possible low DP expression on the cells crossmatched. Patient DT's crossmatch results suggest her apparent 84DEAVx50R antibody is simply an antibody to 84DEAV.

Conclusions

Although their occurrence is very infrequent, it appears that patients can make antibodies to a conjunction of DP-beta and DP-alpha epitopes. When such antibodies are identified, their listing as unacceptable antigens becomes an issue. In these cases, the DPB1 type should be listed, however, as several of the DP beads in the One Lambda LSA panel have atypical DPB1/DPA1 pairings, a DPB1 type should be listed as unacceptable only when the DPB1/DPA1 combination on the positive LSA beads matches the DPB1/DPA1 haplotypes typically seen in the donor population.

Contact

Danny Youngs, Bloodworks Northwest
Email: dannyy@bloodworksnw.org