Acute Heart Transplant Rejection in the Presence of Apparently Weak, Non-Complement- Fixing Donor-Specific Antibodies Detected at the Time of Transplant

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ABSTRACT

Aim: A 66 year old female with an end-stage NYHA class IV inotrope-dependent, ischemic cardiomyopathy received a heart transplant on 9-12-09 from a donor having antigens B13 and DR7. Pre-transplant antibody testing indicated only weakly positive, non-complement-fixing donor-specific antibodies. The patient suffered a cardiac arrest on POD#6 and was resuscitated. She was in cardiogenic shock from allograft failure and subsequently expired on POD#9 of multiorgan failure due to "acute humoral rejection". These antibodies were investigated further.

Methods: Antibodies were evaluated using Labscreen PRA (One Lambda), Labscreen Single Antigen (One Lambda) beads, and C1q Single Antigen (One Lambda) beads, and crossmatching was performed using standard T and B cell CDC and flow cytometry methods.

Results: Pre-transplant antibody studies (Labscreen PRA) had shown the presence of clearly defined antibodies to B27, DR4 and a possible weak anti-DR7. Retrospective Labscreen Single Antigen(SA) (One Lambda) testing revealed antibodies to B13 and DR7, both donor-specific antibodies. MFI values were 2000 for B13 and 5000 for DR7. The pre-transplant crossmatches (CDC and flow cytometry) were negative except for a positive B cell CDC. The patient received blood products on POD#3 and was noted to be in acute renal insufficiency. Crossmatching on POD# 3 showed only a weakly positive T flow crossmatch and negative DSA. However, SA antibody studies on POD#6 showed high MFI values of 19,000 (B13) and 23,000 (DR7), and strongly positive T and B CDC and flow crossmatches. C1q studies of sera from POD#0 and #3 showed the DSA were non-complement fixing. However, by POD#6, the DS antibodies were now clearly complement-fixing. The patient expired on POD#9. Myocardium tissue from the left ventricle taken at autopsy showed positive staining for C4d.
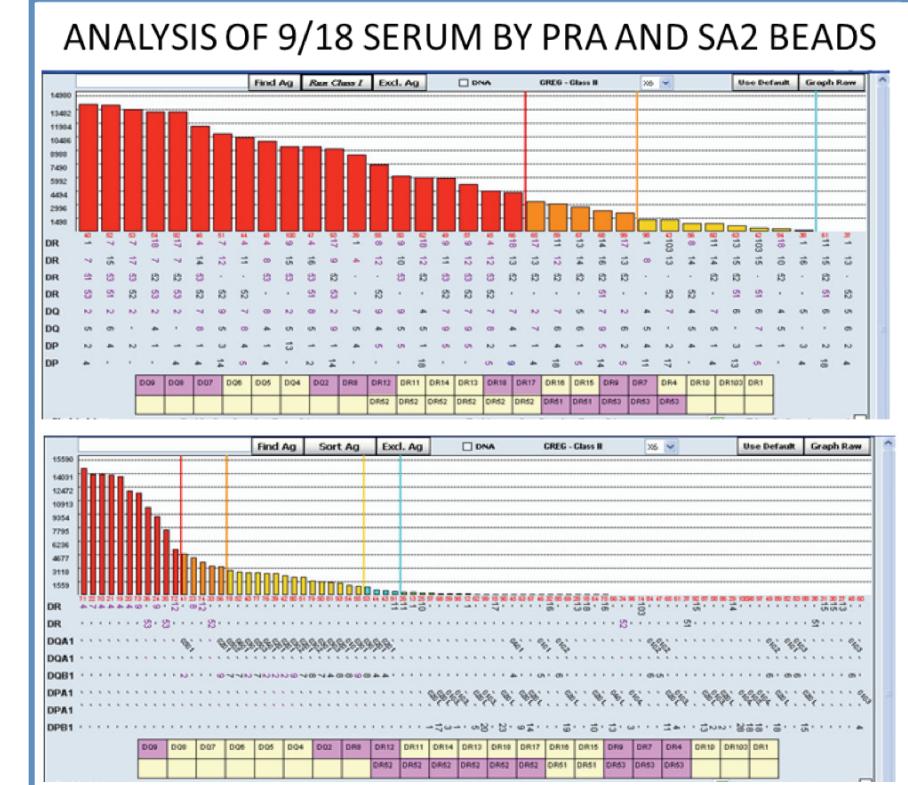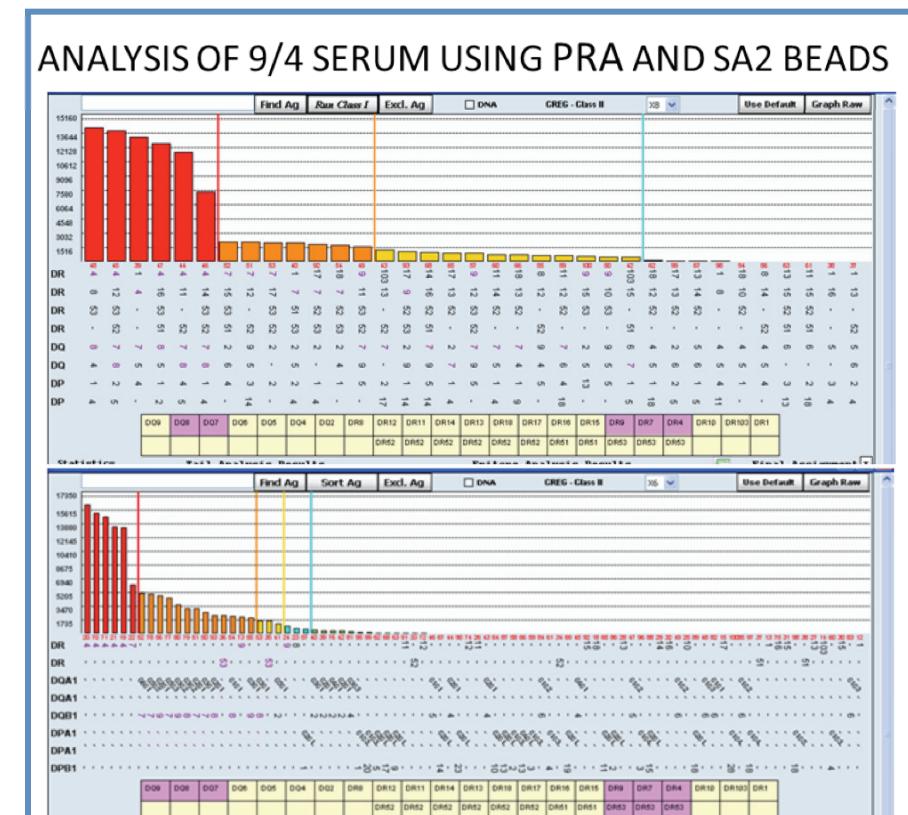
BACKGROUND

- 66 YEAR OLD CAUCASIAN FEMALE
- INITIAL WORKUP 9/4/2009
- DIAGNOSIS: ISCHEMIC CARDIOMYOPATHY, HEPATITIS AND RENAL FAILURE
- 9/12/2009 - HEART TRANSPLANT
- 9/21/2009 – DECEASED: ACUTE HUMORAL REJECTION
- Patient HLA Type: A2,11; B37,55; Bw4,6; DR13,15; DQ6,-; DR51,52
- Donor HLA Type: A11,**26**; **B7,13**; Bw4,6; **DR7**,15; DQ1,**2**; DR51,**53***
- DSA Day of Transplant Anti: DR7 by LABScreen PRA; (DR7 and possible B13 by LABScreen SA, retrospective)
- * In **BOLD**: Mismatched Antigens

Crossmatches Become Strongly Positive at Rejection

	DATE	SEROLOGY		FLOW CYTOMETRY (256 CHANNELS)	
		T (AHG)	B (NIH)	T (POSITIVE MCS = 15)	B (POSITIVE MCS = 30)
PROSPECTIVE DAY 0	12-Sep	NEGATIVE	POSITIVE	NEGATIVE	NEGATIVE
TRANSPLANT			(8+) IgM??		
PO DAY 3	15-Sep	NEGATIVE	NEGATIVE	POSITIVE	NEGATIVE
(TRANSFUSED)				(MCS = 20)	
PO DAY 6	18-Sep	POSITIVE	POSITIVE	POSITIVE	POSITIVE
REJECTION		(6,8+)	(6,8+)	(MCS = 40)	(MCS = 90)

MFI Single Antigen Bead Patterns of DSA DR7 in Pretransplant and Rejection Samples.

ANALYSIS OF 9/4 SERUM USING PRA AND SA2 BEADS

ANALYSIS OF 9/18 SERUM BY PRA AND SA2 BEADS
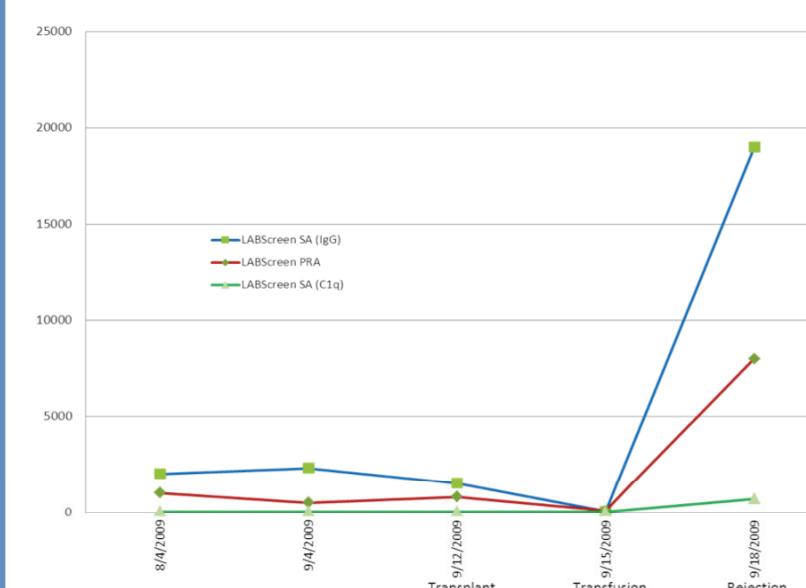
DSA B13 and DR7 Antibodies Change from Weak Non-Complement-Fixing to Strong Complement-Fixing at Rejection

	Sample	At transplant:		Retrospective:			
		METHOD : PRA	MEAN	METHOD : IgG SA	MEAN IgG SA	METHOD : C1q SA	MEAN C1q SA
B13	Date	B13	MFI B13	B13	MFI B13	B13	MFI B13
	8/4/09	NEGATIVE	1000	POSITIVE	2000	NEGATIVE	50
	9/4/09	NEGATIVE	500	POSITIVE	2300	NEGATIVE	50
Transplant	9/12/09	NEGATIVE	800	NEGATIVE	1500	NEGATIVE	50
Transfusions	9/15/09	NEGATIVE	100	NEGATIVE	60	NEGATIVE	50
Rejection	9/18/09	**POSITIVE**	**8000**	POSITIVE	19000	POSSIBLE	700

	Sample	METHOD : PRA		METHOD : IgG SA		METHOD : C1q SA	
DR7	Date	DR7	MFI DR7	DR7	MFI DR7	DR7	MFI DR7
	8/4/09	POSITIVE	3000	POSITIVE	5000	NEGATIVE	20
	9/4/09	NEGATIVE	2000	POSITIVE	6000	NEGATIVE	20
Transplant	9/12/09	POSITIVE	3550	POSITIVE	5000	NEGATIVE	20
Transfusions	9/15/09	NEGATIVE	300	NEGATIVE	1000	NEGATIVE	20
Rejection	9/18/09	**POSITIVE**	**13000**	POSITIVE	23000	**POSITIVE**	**4000**
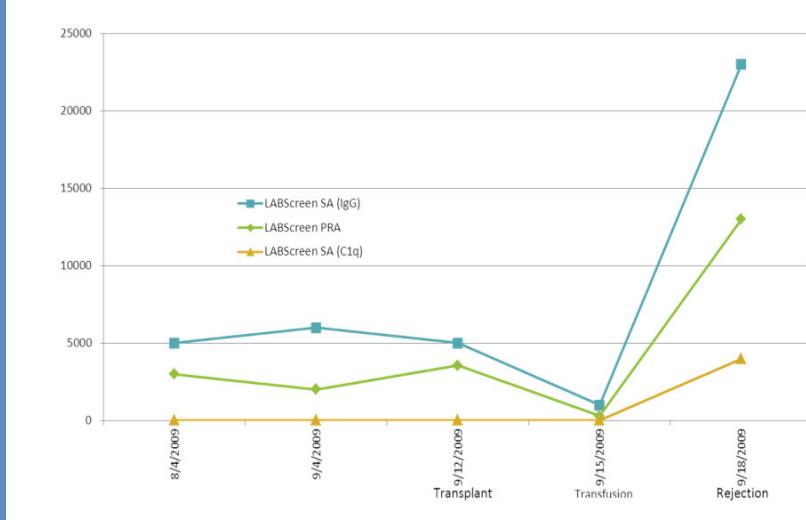
- BOLD: DSA
- MFI cutoff = 2000
- PRA: Labscreen PRA (One Lambda)
- IgG SA: Labscreen Single Antigen(One Lambda)
- C1q SA: C1q Single Antigen assay indicating complement–fixing antibodies (One Lambda)

DSAs Change from Non-Complement-Fixing at Transplant to Complement-Fixing at Rejection

MFIs of DSA B13

MFIs of DSA DR7

SUMMARY

- A putative "weak antibody" (as measured by MFI) at the time of transplant can be a significant risk to transplant outcome.
- A non-complement-fixing antibody can appear later as a complement fixing antibody, i.e. become C1q Positive
- Both types of antibodies can rapidly strengthen to participate in an acute rejection episode.

CONCLUSION

- DSA with relatively weak MFI values can be a significant risk to both organ and patient survival.
- Non-complement-fixing DSAs also pose a risk and can become expressed as complement-fixing antibodies in an acute immune response to mismatched donor antigens.

Antibody Testing Performed Using LABScreen PRA and LABScreen Single Antigen Beads from One Lambda, Inc. (Canoga Park, CA)