Anti-D in Rh Positive Patient with Warm-Reactive Autoantibody: Is it autoantibody or alloantibody?

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Patient History

- 73 y/o African-American female presenting with GI Bleed
- Previously transfused at the referring facility 5 months ago with packed red cells
- History of anti-E, identified at the facility
- Sample referred for
  - resolution of antibody screen, now positive with all cells tested,
  - evaluation of positive DAT, and
  - 2 unit crossmatch

IRL Results

- Patient is group O, Rh positive (no discrepancies)
- DAT is positive

PS	2+	IgG	2+	C_3	0

	PeG IAT		LISS 37C	LISS IAT		Eluate PeG-IAT	LW PeG-IAT
SI	2+		0	1+		3+	0
SII	4+		0	3+		3+	0
SIII	1+		0	1+w		3+	0
EGA Auto	1+s		NT	NT		3+	0

Warm-reactive autoantibody shown to be present in plasma and eluate.

Additional Results

- Phenotype: D+C-E-c+e+; K-; Fy(a-b-); Jk(a+b+); S+s+
- Allogeneic adsorption (rr cell) performed to remove WAA reactivity
- Identified in adsorbed plasma:
  - Anti-E (3+)
  - Anti-C (1+)
  - Anti-D (1+)

The patient is RhD positive.

Is the anti-D in the plasma autoimmune or alloanti-D?

Alloimmune vs Autoimmune anti-D

- Alloimmune anti-D produced by individuals with partial D antigen does not react with the individual's own cells.
  - Autoantibody with a broader specificity was detected in the plasma.
  - The anti-D identified in the plasma was weak; testing this antibody with the patient's cells may be misleading, especially if the cells have reduced antigen expression.
- Autoanti-D can be removed by adsorption with D negative allogeneic cells or the patient's red cells (autoadsorption). Alloimmune anti-D will not be adsorbed and would remain in the plasma.
  - Adsorption results can be misleading in cases with weak antibodies. Further adsorption can dilute the plasma, with the potential of the antibody becoming undetectable.

Other options?

- **Molecular testing**
  - Results not available immediately
  - Cost dependent on how much testing is needed

- **ALBAclone Advanced Partial D Typing Kit**
  - Used to classify partial D types and weak D types 1 and 2
  - Test kit contains 12 IgG anti-D reagents derived from cultures of human/mouse heterohybridomas
  - Reaction profile of these antibodies with the test cells may point to a partial D or weak D classification. Further investigation is needed if the results do not fit into a reaction profile.

Partial D Categories

- Partial D phenotypes were originally classified serologically into 7 categories based on the interaction of the rbcs and sera of other D category members. The presence of low incidence antigen markers also helped in the identification.
  - DVa cells are D^{w} positive (Wiel)
  - DIVa cells are Go^{a} positive (Gonzales)
- Studies with monoclonal anti-D revealed even more patterns, which recognized 9 different epitopes of the D mosaic. Subsequently these nine epitopes were split and sub-split to 30 epitopes using hundreds of monoclonal antibodies.
- New partial D phenotypes discovered are no longer given category numbers; now denoted by upper case letters (DFR, DHAR, etc.)
- Genetics: There are 90 known alleles that encode partial D; not all can be serologically distinguished. Genotyping is definitive in most cases.

ALBAclone Partial D Typing Kit Reaction Profile

Anti-D Cell Line	Weak D Type 1 and 2[ø]	DII & DNU	DIII	DIV	DV[*]	DCS	DVI	DVII	DOL	DFR	DMH	DAR[†]	DAR-E	DHK[‡] & DAU-4	DBT	Ro[Har §]
LHM76/58	+	+	+	+	+/0	+	0	+	+	+	+	+	0	0	0	(+)/0
LHM76/59	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0
LHM174/102	(+)/0	+	+	0	0	+	0	+	0	0	+	0	0	0	0	0
LHM50/2B	+	+	+	+	+	+	0	+	+	+	+	+	+	+	0	0
LHM169/81	+	+	+	0	0	+	0	+	+	+	+	0	0	0	0	0
ESD1	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0
LHM76/55	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0
LHM77/64	+	0	+	0	+	+	+	+	+	+	+	+	+	+/0	0	0
LHM70/45	(+)/0	+	+	0	0	0	0	+	0	0	0	0	0	0	0	0
LHM59/19	+	+	+	+	+	+	0	0	0	0	(+)	0	(+)	+	+	0
LHM169/80	+	+	+	+	+	+	0	+	+	+	+	+	+	0	0	0
LHM57/17	+	+	+	+	+	0	0	+	+	0	+	+	0	0	+	0

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Results (EGA-treated patient cells)

Patient	Profile for DIV
1	4+
2	0 ✓
3	0 ✓
4	4+
5	0 ✓
6	0 ✓
7	0 ✓
8	0 ✓
9	0 ✓
10	4+
11	4+
12	4+

1	+
2	0
3	0
4	+
5	0
6	0
7	0
8	0
9	0
10	+
11	+
12	+

DIVa

- There are 5 types of DIV cells
  - DIVa associated with ce haplotype; Go(a+)
  - DIVb associated with Ce or cE haplotypes
  - DIV type 3, associated with Ce
  - DIV type 4, associated with Ce
  - DIV type 5, ???
- Only DIVa and DIVb are known to produce anti-D
- Patient cells were tested with anti-Go^{a} and found to be Go(a+).
- DIVa occurs predominantly in Blacks
- Red cells of DIVa individuals typically show enhanced expression of the D antigen and react strongly with most examples of human anti-D

Is the anti-D auto- or alloantibody?

❑ “Mimicking” autoanti-D can be present in D positive or D negative individuals, and presumably people with partial D antigen as well.

The real question: Do we transfuse with Rh positive or Rh negative rbcs?

❑ Since we have shown that this patient has the potential to produce anti-D by characterizing her cells as DIVa, we will treat this anti-D as alloantibody, and recommend transfusion of D-C-E- red cells.

❑ We were lucky – if the partial D typing results were not conclusive, molecular testing would be needed to resolve the question.

Wishing Everyone Good Luck!