Anti-IB In An A_1B Patient With Rectal Cancer

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Introduction

- 61-year-old male admitted for surgical resection of rectal cancer.
- Patient's ABORh front type was AB positive
- Unexpected reactivity with B cells in the reverse type on multiple specimens
- Suspected acquired B phenomenon, but *ABO* genotype identified *A1* and *B* alleles (predicted A_1B phenotype)
- Further testing identified an anti-IB with underlying anti-I reactive only at 4°C.

Materials and

- ABORh typing was performed by commercial monoclonal anti-A, anti-B, and anti-D antisera, and commercial A_1 and B red blood cell (RBC) reagents at Immediate Spin (IS).
- The antibody detection test was performed by solid phase red cell adherence.
- Additional testing with the patient's plasma was performed using commercial reagent RBC, volunteer donor RBC, and cord RBC at IS, room temperature (RT), and 4°C incubation.
- Adsorption studies were performed using ficin treated donor RBC incubated for 30 minutes at 4°C.
- Titrations were performed using type O and type B RBC at RT and 4°C incubation.
- A_1 typing was performed with commercial anti-A_1 lectin.
- ABO genotyping was performed at an outside laboratory.

Results

- The patient's antibody detection test was negative.
- The antibody showed strongest reactivity at IS with B and A_2B adult RBC.
- Negative reactions with cord RBC at IS suggested anti-IB specificity.
- Negative reactivity with A_1B RBC may be due to weaker expression of B antigen on A_1B RBC compared to A_2B RBC.
- Reactivity at 4°C with O and A_1B RBC was attributed to anti-I.

Results

Figure 1: Front Type

Front type determined by commercial reagents at immediate spin. Genotype determined by molecular methods at outside lab. Patient RBCs also typed 4+ with type A patient plasma (not shown). Reactivity suggestive of A_1B phenotype.

	Anti-A	Anti-B	Anti-A,B	Anti-D	Monoclonal Control	Anti-A_1 Lectin
Patient RBC	4+	4+	4+	4+	0	3+
Probable Genotype: *ABO*A1 / ABO*B*						

Figure 2: Reverse Type

Reverse type tested by commercial reagent RBC, donor unit RBC and patient cord RBC suspended to approximately 3-4% suspension and tested at immediate spin, room temperature incubation and incubation at 4°C. No reactivity observed with A_1, A_2 or A_1B RBC at IS or RT suggests anti-B reactivity. Reactivity at 4°C was stronger with B and A_2B adult cells, but reactivity was also noted at 4°C with type O and A_1B adult cells which suggests anti-IB and an underlying anti-I. The reactivity with B cord cells at 4°C may be due to anti-IB due to expression of B antigen and weak I antigen. (NT=Not Tested)

	A_1 cells	A_2 cells	B cells	O cells	A_1B cells	A_2B cells	O cord cells	B cord cells	A_2B cord cells	Patient cells
IS	0	0	2+	0	0	2+	0	0	0	0
RT	NT	NT	2+	0	0	2+	NT	NT	NT	W
4°C	NT	NT	4+	1+	2+	4+	0	1+	0	1+

Figure 3 – Cold alloadsorption of patient plasma

Aliquots of patient plasma adsorbed with type O and type B donor RBC. Adsorption with B RBC removed all reactivity. Anti-B reactivity remained after adsorption with type O"RBC.

	A_1 cells		A_2 cells		B cells		O cells	
	IS	RT	IS	RT	IS	RT	IS	RT
4°C adsorption with type B RBC	0	0	0	0	0	0	0	0
4°C adsorption with type O RBC	0	0	0	0	0	3+	0	0

Figure 4: Titration with B cells

Titration with type B, I positive RBC and type B, I negative RBC at RT and 4°C incubation. Higher titer with B,I+ cells suggests anti-IB (B,I+ titer = 4 and B,I- titer = 2).

Cells	Incubation Time/Temp	1:1	1:2	1:4	1:8	1:16	1:32
B, I+	15' RT	1+	0	0	0	0	0
	30' 4°C	3+	2+	1+	0	0	0
B, I-	15' RT	0	0	0	0	0	0
	30' 4°C	2+	1+	0	0	0	0

Figure 5 – Titration with O cells

Titration with type O, I positive RBC and type O, I negative RBC at RT and 4°C incubation. Anti-I detected at 4°C (titer = 1).

Cells	Incubation Time/Temp	1:1	1:2	1:4	1:8	1:16	1:32
O, I+	15' RT	0	0	0	0	0	0
	30' 4°C	1+	0	0	0	0	0
O, I-	15' RT	0	0	0	0	0	0
	30' 4°C	0	0	0	0	0	0

Conclusion

- Acquired B phenomenon was ruled out by molecular testing.
- Possible B variants could not be excluded due to unavailability of high-resolution testing, but would be unlikely with a strong 4+ reaction with anti-B.
- There have been other reports in the literature of anti-IB in type B and A_1B patients, but these are rare.
- The anti-IB in this patient is likely an autoantibody and is not expected to cause hemolytic transfusion reactions, however, an "O" RBC requirement was initiated out of an abundance of caution.
- The patient did not require transfusion, and the anti-IB was no longer detected 3 months post-operatively.