


Characterizing Unidentified Antibodies

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Objectives


- Define the term Unidentified Antibody as used by the IRL
- Examine the techniques used by the IRL to help characterize the unidentified reactivity
- Review two case studies outlining the process for further characterization of previously unidentified antibodies
- Discuss transfusion recommendations for patients with unidentified antibodies

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Distinguishing Significant Antibodies

Trying to distinguish between significant and insignificant antibodies requires experience, persistence, and a variety of techniques. Given apparent nonspecific or broad reactivity, how do we proceed?

- Examine the patient's history!
- Is there any variation in reaction strength?
- What is the result of the DAT?
- What is the result when testing different enhancement methods?
- What are the results when testing enzyme or chemically treated cells?

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Distinguishing Autoantibodies from Alloantibodies

It can be difficult to determine whether broad specificity in a patient's serum is due to autoantibody, particularly when the DAT is positive.

- Compare the strength of the DAT to the strength of the antibody in the serum.
- What does the eluate show?
- What is the result of the DAT?
- If the patient has not been recently transfused, try an autoadsorption.
- Test the patient's serum against their DAT negative autologous red cells



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Case Study 1

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Patient History

- Our laboratory received a sample on a 70-year-old Caucasian in November of 2018. This patient was hospitalized for sepsis and was last transfused in 2016. A history of a warm autoantibody was provided to us from another facility.
- The patient is A Negative, DAT is negative, and a common red cell phenotype was performed.

ABO/Rh	Anti				Cells				DAT	
	A	B	D	Cont	A	B	D	IS	PS	
IS	4+	0	4+	0	0	0	0	3+	IS	0
37(AI)			0✓	0✓				RT		0✓

Red Cell Phenotype	C	E	e	a	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	Le ^a	Le ^b	Pl
	Phenotype	0	0	4+	4+	0✓	3+	0✓	4+	0	3+	3+	0	0✓	3+	0	3+



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The Patient's Next Admission

- On August 13, 2019 the patient was hospitalized for "pancytopenia". Her Hgb at admission was 6.9 and two units of blood were ordered.
 - Washed Red Blood Cells were ordered due to "allergic" reactions to previous transfusions.
- There was no change in the reactivity of the patient's serum from the 2018 to the 2019 sample.
- Two units of D-, C-, E-, K-, Fy(a-), Jk(a-), S- units were washed and sent to the facility for transfusion.
- Additional phenotypically similar Washed RBCs were sent for transfusion until 08/24/2019, when a transfusion reaction was called.

Transfusion Reaction Workup

- The patient experienced vigorous shaking, hypertension, chills, and nausea.
- The pre-transfusion DAT was negative and the post-transfusion DAT was 1+ on the hospital's workup.
- Samples were sent to the IRL for a transfusion reaction investigation.
- The IRL obtained a negative DAT on the pre- and post-transfusion samples (note that the post-transfusion sample was collected at a later time than the hospital's sample).
- There was no change in the reactivity of the serum.

Characterization of the Unidentified Antibody

The patient's serum was tested with cord cells, null cells, and cells that lack high prevalence antigens which are not affected by cell treatments.

	D	C	E	+C	M	H	S	I	Lep	Lep	Le ^a	Le ^b	Le ^x	K ^a	K ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	S	Other	
DEX-RHD 12 IRCO-0918	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Rh-17 2+
444C	+	+	0	0	+																	W+
IRK-RSD-8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	W+
IRK-0918	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	W+
IRK-RSD 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	W+
IRCO-0918	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	W+
IRK-RSD 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	W+
IRCO-0918	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	W+
IRK-RSD 11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	W+
IRCO-0918	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	W+
333C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	W+
447C	+	+	0	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	W+
600P	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	W+
602C	0	0	0	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	W+

Characterization of the Unidentified Antibody

The negative Lu(a-b-) cell was also AnWj-. The AnWj antigen is weak on dominant Lu(a-b-) cells. Cord cells were nonreactive. Cord red blood cells do not express the AnWj antigen. At this time, additional AnWj- cells were tested to confirm the specificity.

The AnWj antigen is the receptor for *Haemophilus influenzae* and is carried on the CD44 proteoglycan. The AnWj- phenotype is usually the result of transient suppression of the antigen, which may be long term. The clinical significance of Anti-AnWj is variable, and it does not cause HDFN.



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Determining Clinical Significance

Because of the severe transfusion reaction, a monocyte monolayer assay (MMA) was performed.

			IAT	MMA
Patient Serum	AnWj+ Red Cells		1+	33-5%
Patient Serum	AnWj+ Red Cells	Fresh Normal Serum	1+	35-5%
Patient Serum	AnWj- Red Cells		0	1.3%
Patient Serum	AnWj- Red Cells	Fresh Normal Serum	0	1.3%

MMA results are expressed as % reactivity of monocytes. Analysis suggests that results greater than 5% can be associated with overt transfusion reactions in patients who receive incompatible RBCs.



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Transfusion

The patient was transfused successfully with AnWj- red cells (not washed). Because of the extraordinary rarity of this blood type, the search also included Lu(a-b-) cells. These RBCs often possess weak expression of the AnWj- antigen. An international search was conducted by the American Rare Donor Program. Products were located in England; however they were not imported.



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Case Study 2

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Patient History

- Our laboratory received a sample on a 34-year-old Caucasian female in July of 2020. This patient was hospitalized with cystic fibrosis and had a hemoglobin of 5.6. They had previous transfusions but none in the prior three months. The hospital reported panreactivity, positive DAT, cold autoantibody reactivity, and nonspecific reactions.

ABO/Rh	Anti-Cells						DAT					
	A	B	AB	D	Cross	A ₁	B ₁	PS	IgG	C	CT	
IS	4+	4+	4+	3+	0	1+	0	IS	4+	3+	0	
	RT										3+	0

Red Cell Phenotype	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	Le ^a	Le ^b	P1	A1	
	4+	0	4+	4+	0	3+	2+	2+	3+	0	0	3+	3+	0	3+	3+	0	0	0

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Initial Antibody Panel

	D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	Le ^a	Le ^b	P1	A1	PEG IAT	
Imm-20-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Imm-20-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Imm-20-3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Imm-20-4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
Imm-20-5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
Imm-20-6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
Imm-20-7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Imm-20-8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Imm-20-9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Imm-20-10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Imm-20-11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Imm-20-12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Imm-20-13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Imm-20-14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Imm-20-15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Imm-20-16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Imm-20-17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Imm-20-18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1+
Auto	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1+

The initial antibody panel was performed at IS and PEG/IAT. Reactivity was noted at PEG/IAT with all reagent red cells except those that were e-.

EGTA Treated Auto Cells			DAT Non Auto Cells + Serum	
DAT IgG	CT		PEG/IAT	CT
IS	0	0	1+	0


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Drug Study Results

Drug study (Piperacillin + Tazobactam):

Test	60-37°C	Saline IAT
Patient Serum + Drug	0	2+
Patient Serum + Drug + Normal Serum	0	1+
Patient Serum + PBS	0	0
Patient Serum + PBS + Normal Serum	0	0
Drug + Normal Serum	0	0
PBS + Normal Serum	0	0

- The patient's serum is tested against a group O reagent cell with the addition of a solution of drug. A complement source is also added. Controls are tested to ensure that the normal serum does not react in the presence of the drug, and that no nonspecific reactivity is noted.

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Discussion

We now know our patient's serum contains an antibody reactive in the presence of the drug Zosyn®.

Piperacillin is known to cause Drug-Induced Hemolytic Anemia. The symptoms include acute intravascular hemolysis with hemoglobinuria. Once a drug-dependent antibody of this type has formed, severe hemolytic episodes may occur after exposure to a very small quantity of the drug.

The Tazobactam component of the drug is known to cause non-immunologic protein adsorption and a positive DAT. Hemolytic anemia rarely occurs with this mechanism.

The drug must be discontinued, and the patient's medical record should be updated to include this information.

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Serology Resolution

What about the apparent anti-e specificity in the serum?

In 2010 George Garretty's laboratory investigated anti-e-like reactivity in a patient sample submitted for DIHA investigation. A piperacillin antibody was identified, and the serum also reacted with all e+ cells tested.

Their conclusion was that piperacillin antibodies may show a preference to e+ red blood. In addition, drug-anti-drug complexes in the serum may mimic anti-e. It is important not to confuse piperacillin-induced hemolytic anemia with a hemolytic transfusion reaction or with autoimmune hemolytic anemia.

Bandara, Mahesh, et al. "Piperacillin-Induced Immune Hemolytic Anemia in an Adult with Cystic Fibrosis." *Case Reports In Medicine*, vol. 2010, 2010, doi:10.1155/2010/161454.

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Conclusions

Investigating complex antibody cases can be both frustrating and rewarding. Although on paper these cases follow a logical progression, the testing represents hours of investigation and hard work by the IRL.

It is important not to dismiss reactivity as something insignificant. Consult with your Medical Director and the patient's Physician to assess patient transfusion needs when an unidentified antibody is present.



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