

# Warm Autoantibody Workups

Daniel Held, MHS, MT(ASCP)SBB  
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# Objectives

- Describe the common clinical signs and routine laboratory testing for autoimmune hemolysis.
- Describe the process of determining which red blood cells to select for adsorption of warm autoantibodies.
- Discuss the problem solving techniques involved in AIHA.





# Introduction

- Autoimmune Hemolytic Anemia (AIHA)
  - Response to RBCs
  - Shortened survival of RBCs → anemia
  - Serological problems in transfusion services



# AIHA Causes

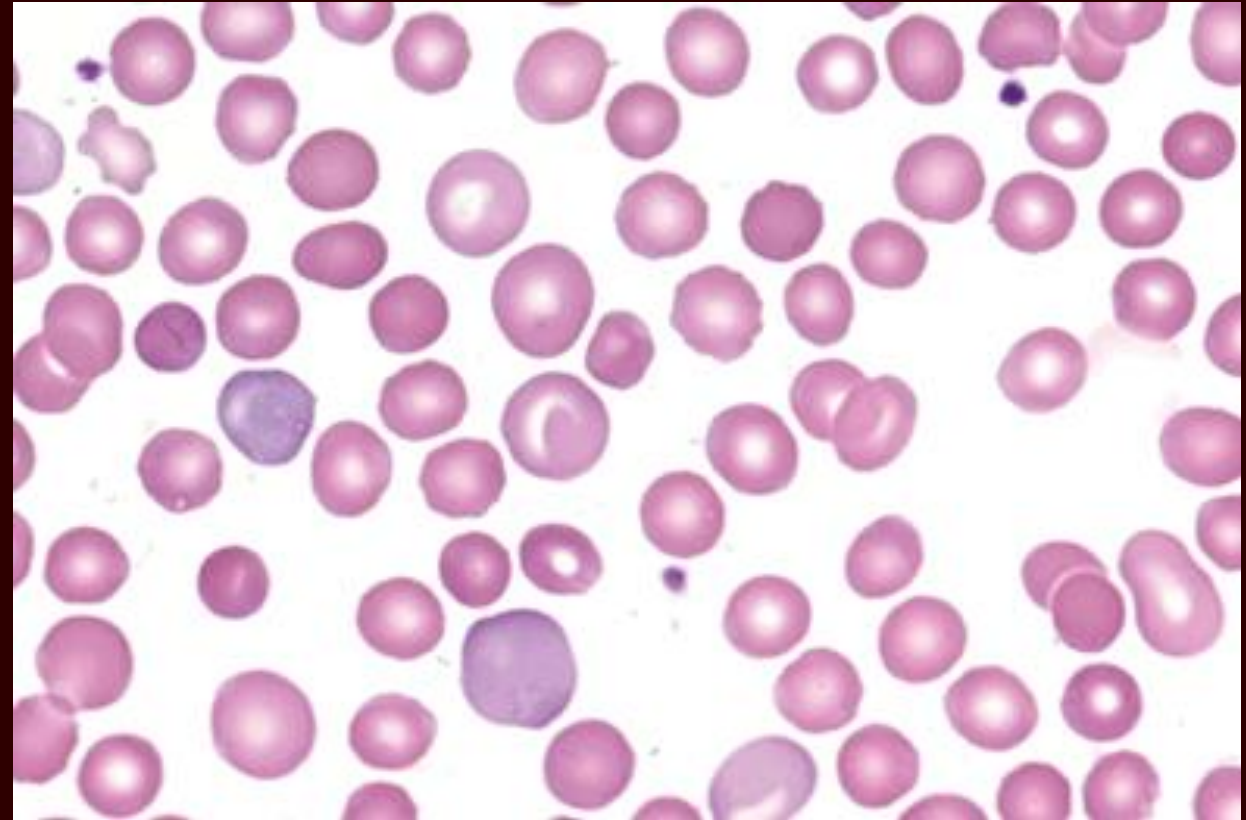
- Primary (30%) or secondary (70%)
- AIHA associated with:
  - Immune dysfunction diseases
  - Neoplasms of the immune system
  - Immune deficiency states
  - Current or post-infectious periods





# AIHA Diagnosis

- Based on lab values and clinical findings
- Anemia (Hgb <7 g/dL)
- Blood smear
  - Polychromasia
  - Spherocytes
  - Fragmented cells



# AIHA Diagnosis

- Other common lab values/clinical findings:
  - Increased unconjugated bilirubin
  - Increased LDH
  - Decreased haptoglobin
  - Possible increased plasma hemoglobin
  - Possible visible hemoglobinuria
  - Decreased hemoglobin and hematocrit
  - Increased reticulocyte count





# Classification of AIHA

- Warm AIHA – IgG and/or C3
- Cold agglutinin syndrome (CAS) – IgM and C3
- Mixed-type AIHA – IgM, IgG, and C3
- Paroxysmal cold hemoglobinuria (PCH) – biphasic IgG and C3
- Drug-induced AIHA – IgG and/or C3



# WAIHA Pathophysiology

- **Extravascular hemolysis**
  - Most common
  - IgG and/or C3 coated RBCs removed in liver/spleen
- **Intravascular hemolysis**
  - Classical complement cascade activated
  - Hemoglobinemia → haptoglobinemia → hemoglobinuria





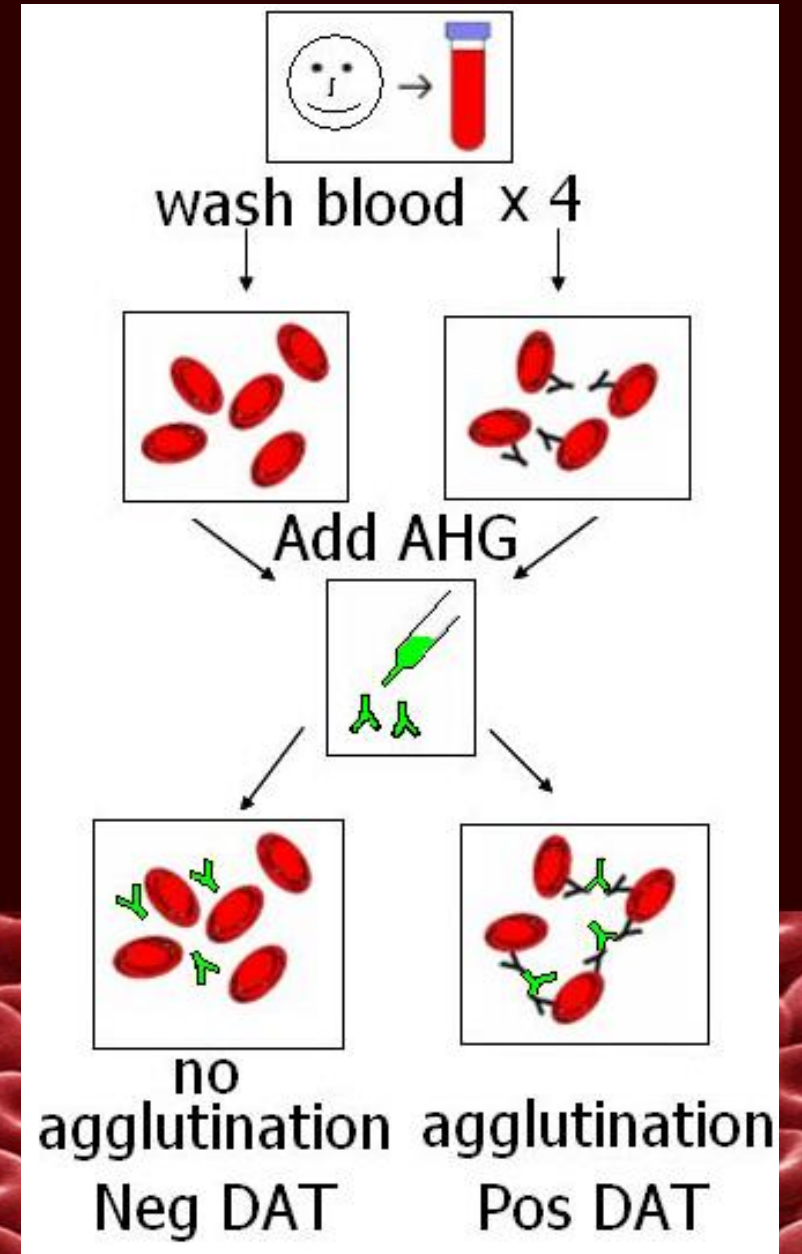
# WAIHA Pathophysiology

- IgG subclass and C3
  - IgG1/IgG3 > IgG2
  - IgG4 = no hemolysis
  - IgG & C3 = high hemolysis



# WAIHA Serology

- Positive direct antiglobulin test (DAT)
  - IgG and C3 (67%)
  - IgG alone (20%)
  - C3 alone (13%)
- *In vivo* attachment





# WAIHA Serology

- Positive DAT → Elution
  - Removal of IgG from RBC surface
  - Eluate tested against reagent cells
- Eluate from AIHA displays panagglutination
  - Occasionally may look like autoantibody to specific antigen (i.e. autoanti-e)



# WAIHA Serology

- Warm autoantibody quantity
  - Low titer:
    - DAT+ but negative antibody screen
    - WAA is completely adsorbed onto patient's RBCs
    - Crossmatch compatible or least-incompatible
  - High titer:
    - DAT+ with positive antibody screen (panagglutination)
    - WAA has “spilled over” in the serum
    - Determine if alloantibodies are being “masked” by WAA





# WAIHA Serology

- “Spill over” of WAA into patient’s serum
- Is there alloantibody(-ies) being hidden by WAA?
  - Adsorb WAA out of serum using RBCs to determine

	<b>IS</b>	<b>37C</b>	<b>AHG</b>
SCI	0	0	3+
SCII	0	0	3+
SCIII	0	0	3+
AC	0	0	3+



# WAIHA Adsorptions

- Cell selection for adsorption
  - Ensure cells used DO NOT adsorb alloantibodies
  - Autologous cells – if not transfused within 3 months
  - Allogeneic cells – find antigen-matched with patient's phenotype





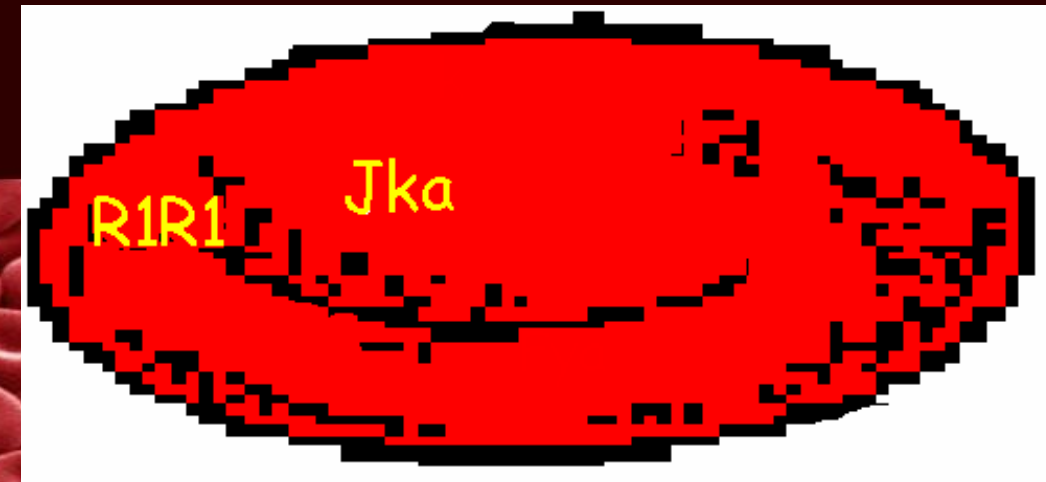
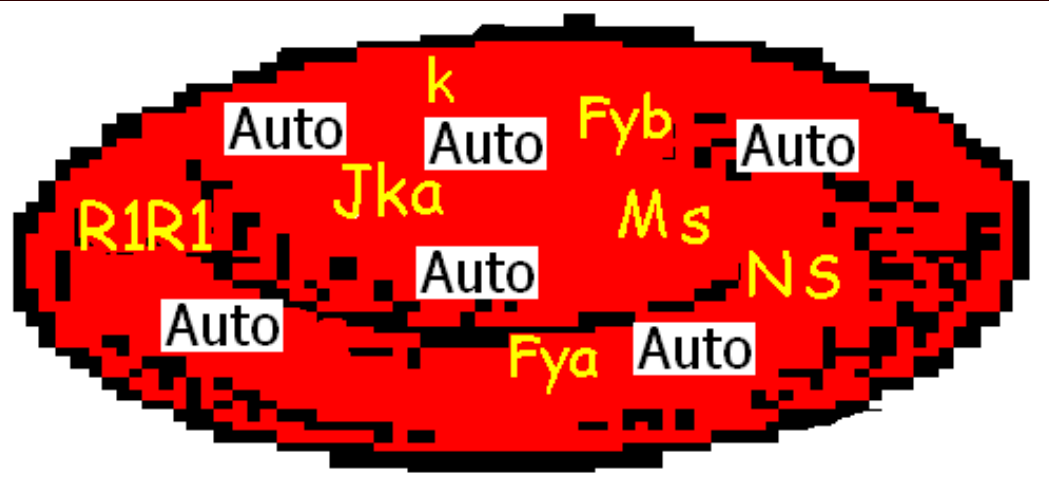
# WAIHA Autoadsorptions

- Autologous cells are coated with WAA IgG
  - Adsorption sites are already blocked
- Remove IgG first before adsorption
  - Gentle heat elution (56°C for 3-5 min)
  - Chemical treatment (chloroquine diphosphate or ZZAP)



# WAIHA Autoadsorptions

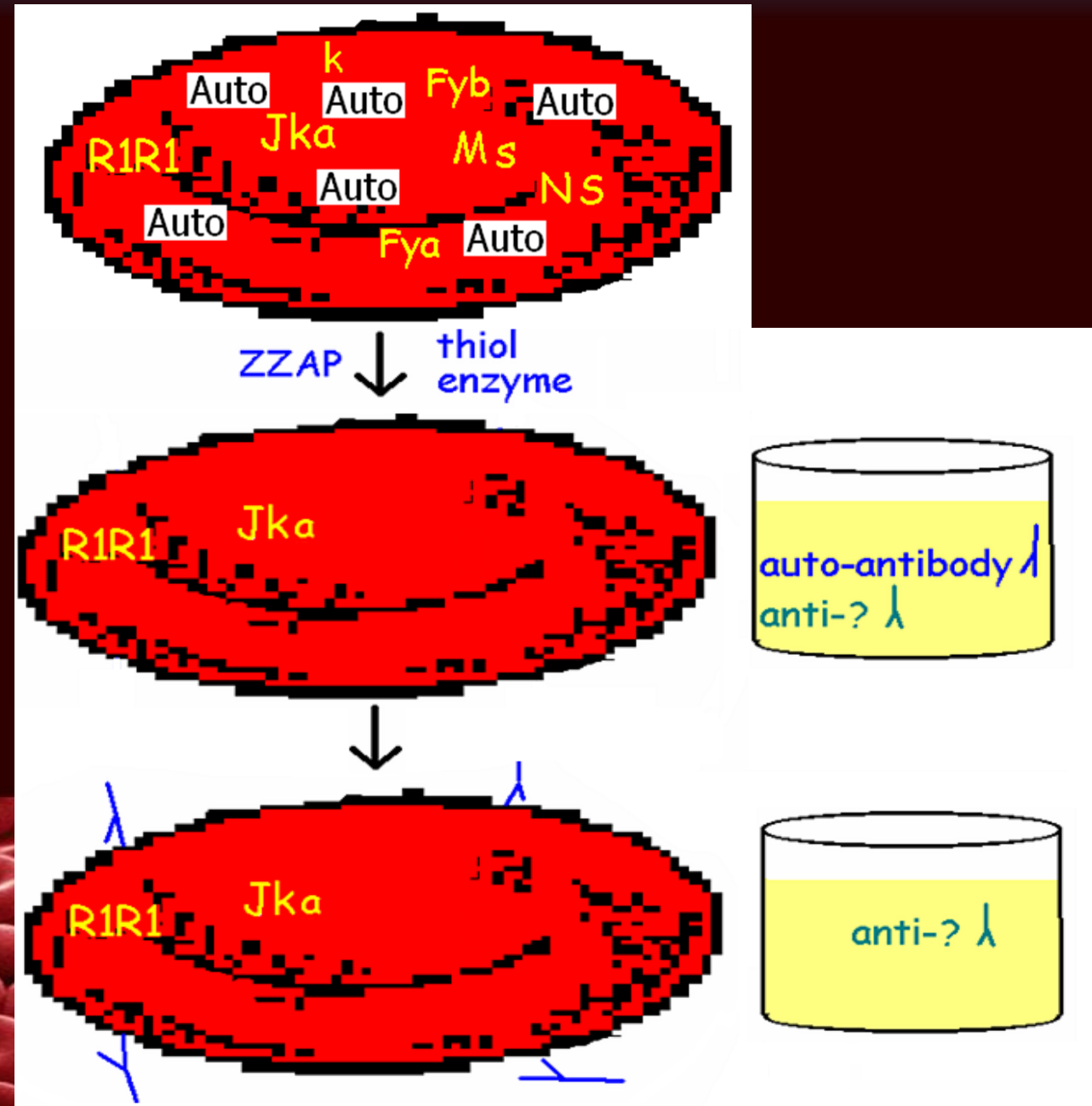
- ZZAP treatment most common
  - Ficin/papain + dithiothreitol (DTT)
  - 1:1 mixture of patient RBCs and ZZAP at 37°C for 30-45 min
  - Removes WAA and destroys K, Duffy, MNS antigens





# WAIHA Autoadsorptions

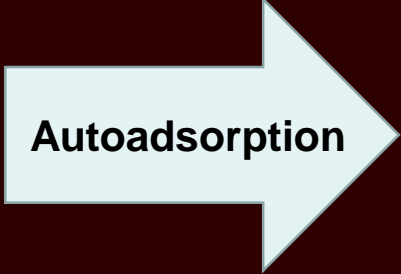
- ZZAP-treated auto cells incubated with serum
- WAA adsorbed onto cells
- Remaining alloantibody(-ies) remain in serum



# WAIHA Autoadsorptions

	IS	37°C	AHG
I	0	0	3+
II	0	0	3+
III	0	0	3+
AC	0	0	3+

Autoadsorption



	IS	37°C	AHG
I	0	0	0
II	0	0	1+
III	0	0	0
AC	0	0	0





# WAIHA Alloadsorptions

- Auto cells not usable in recently transfused patients
  - Transfused cells may adsorb out alloantibodies present
- Find allogeneic donor cells phenotypically identical
- Perform full phenotype on patient
  - Molecular testing best
  - Serological testing more common, but...



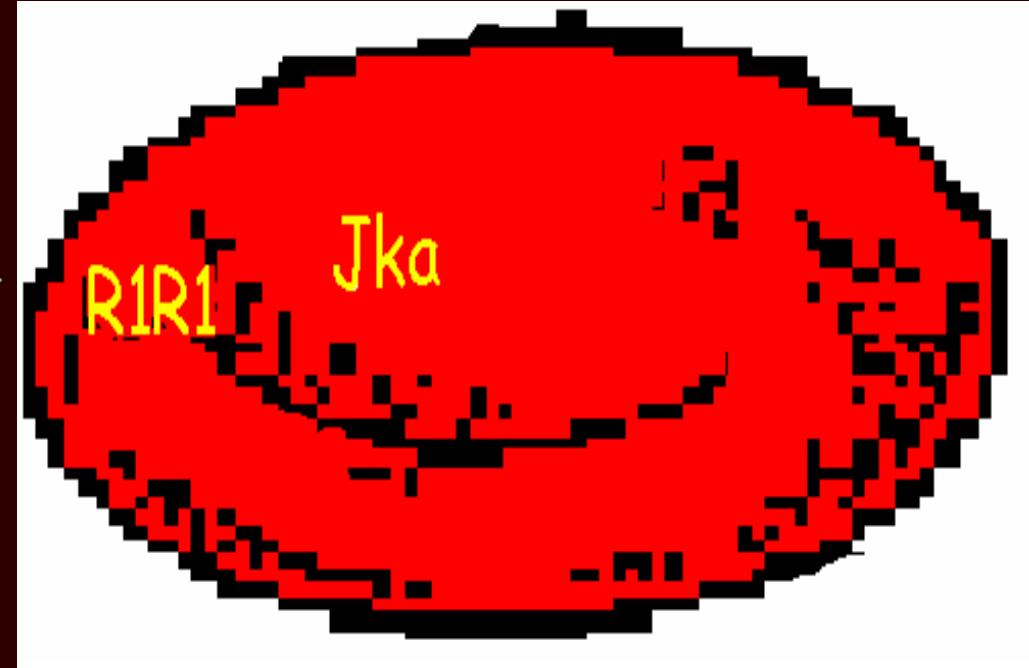
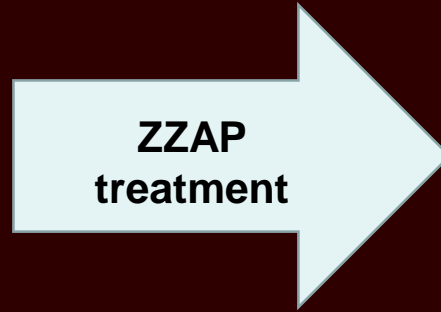
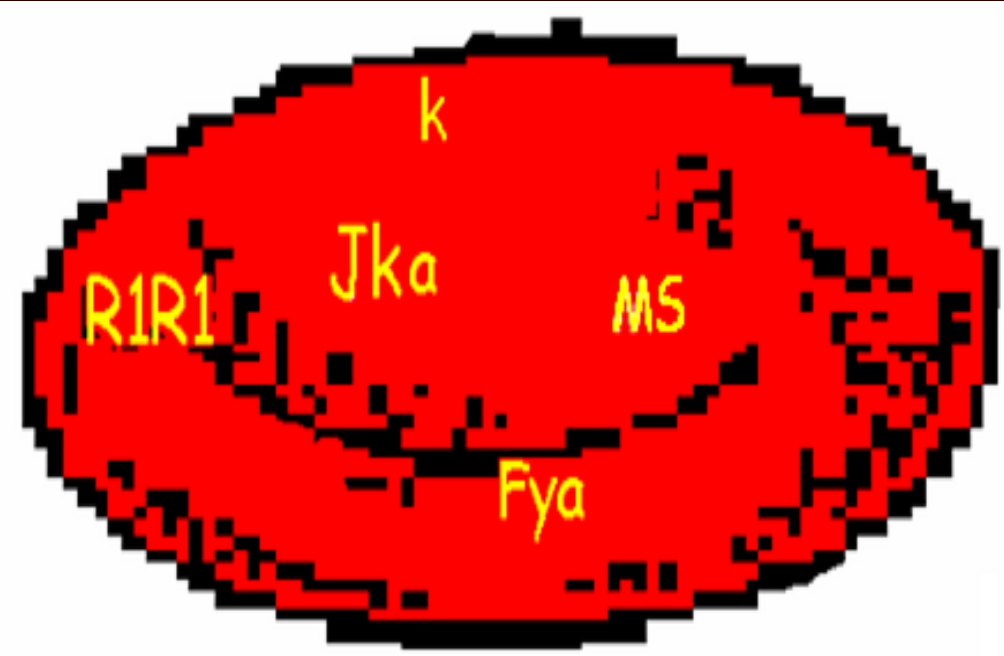
# WAIHA Alloabsorptions

- Serological antigen typings
  - IgM serological typings acceptable (Rh, K, Jk, MNS)
  - IgG serological typings result in false positives (Fy, s)
- If able to obtain Rh and Kidd typings, ZZAP treatment of allogeneic cells can be performed





# WAIHA Alloadsorptions



# WAIHA Alloadsorptions

- If unable to determine Rh and Kidd antigen typings:
  - Massive recent transfusion
  - IgM typing reagent unavailable
- Differential alloadsorption to be performed
  - Multiple alloadsorptions using different donors
  - ZZAP treatment of all donors remains same

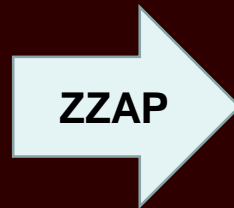




# WAIHA Alloabsorptions

- R1R1, R2R2, rr with one Jk(a+b-) and one Jk(a-b+)

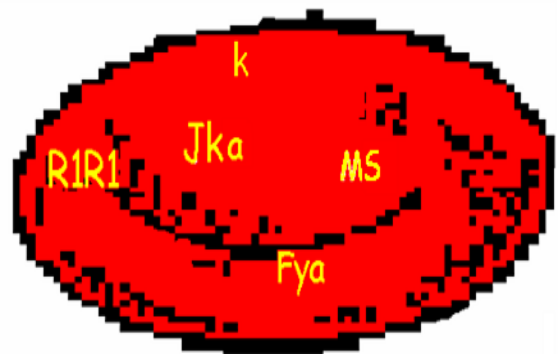
	Rh	MNS	Duffy	Kidd	Kell
R1R1	DCe	MS	Fyb	Jka	kk
R2R2	DcE	Ns	Fyb	Jkb	kk
rr	ce	MSs	Fya	Jkb	kk



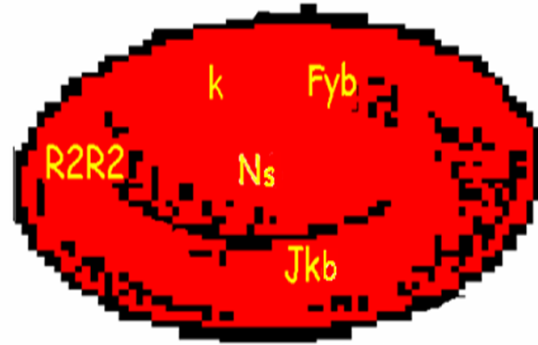
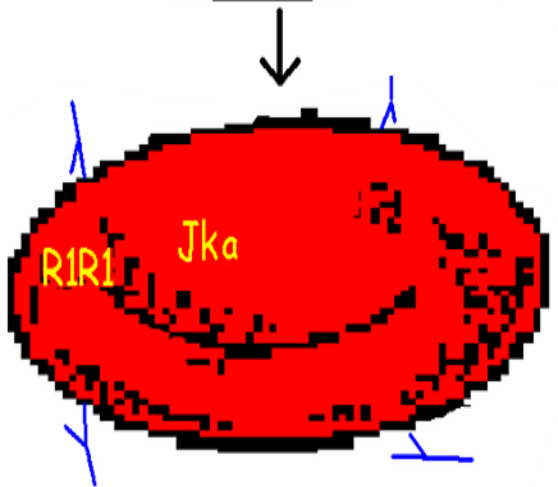
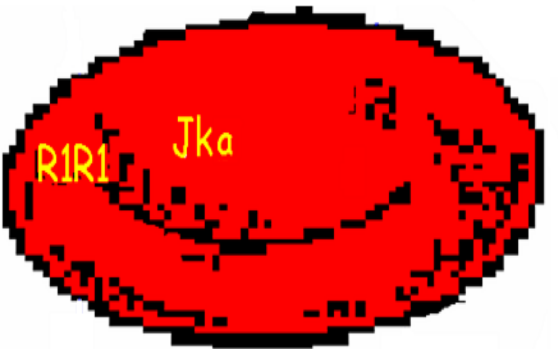
	Rh	MNS	Duffy	Kidd	Kell
R1R1	DCe	<del>MS</del>	<del>Fyb</del>	Jka	<del>kk</del>
R2R2	DcE	<del>Ns</del>	<del>Fyb</del>	Jkb	<del>kk</del>
rr	ce	<del>MSs</del>	<del>Fya</del>	Jkb	<del>kk</del>



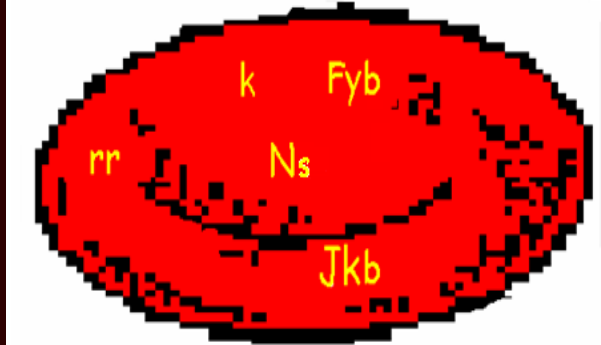
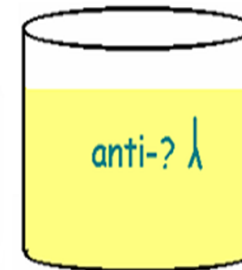
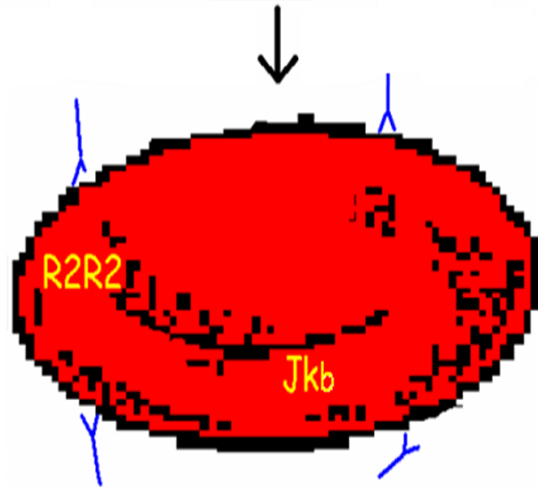
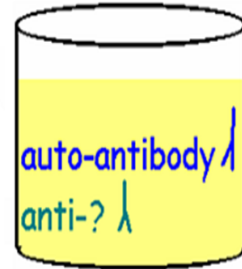
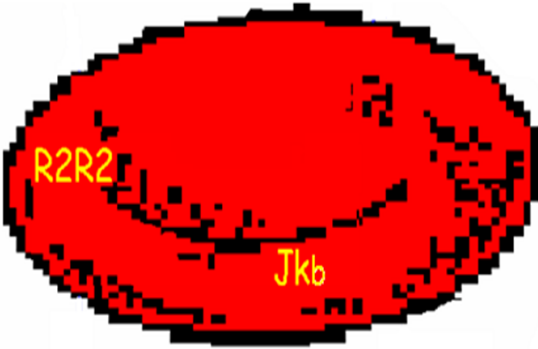
# WAIHA Alloadsorptions



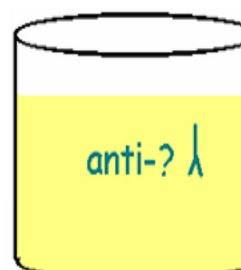
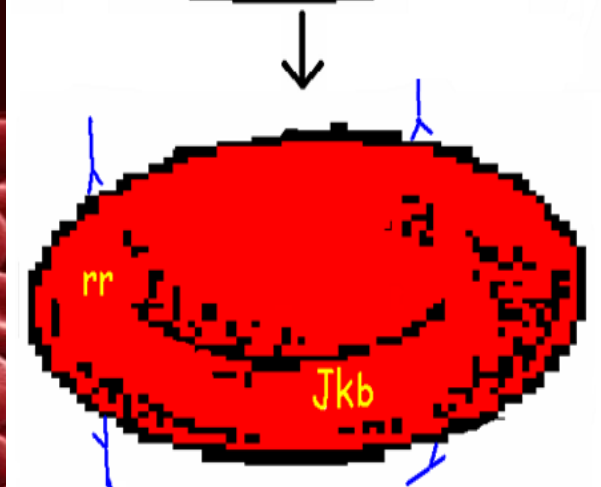
ZZAP ↓ thiol enzyme



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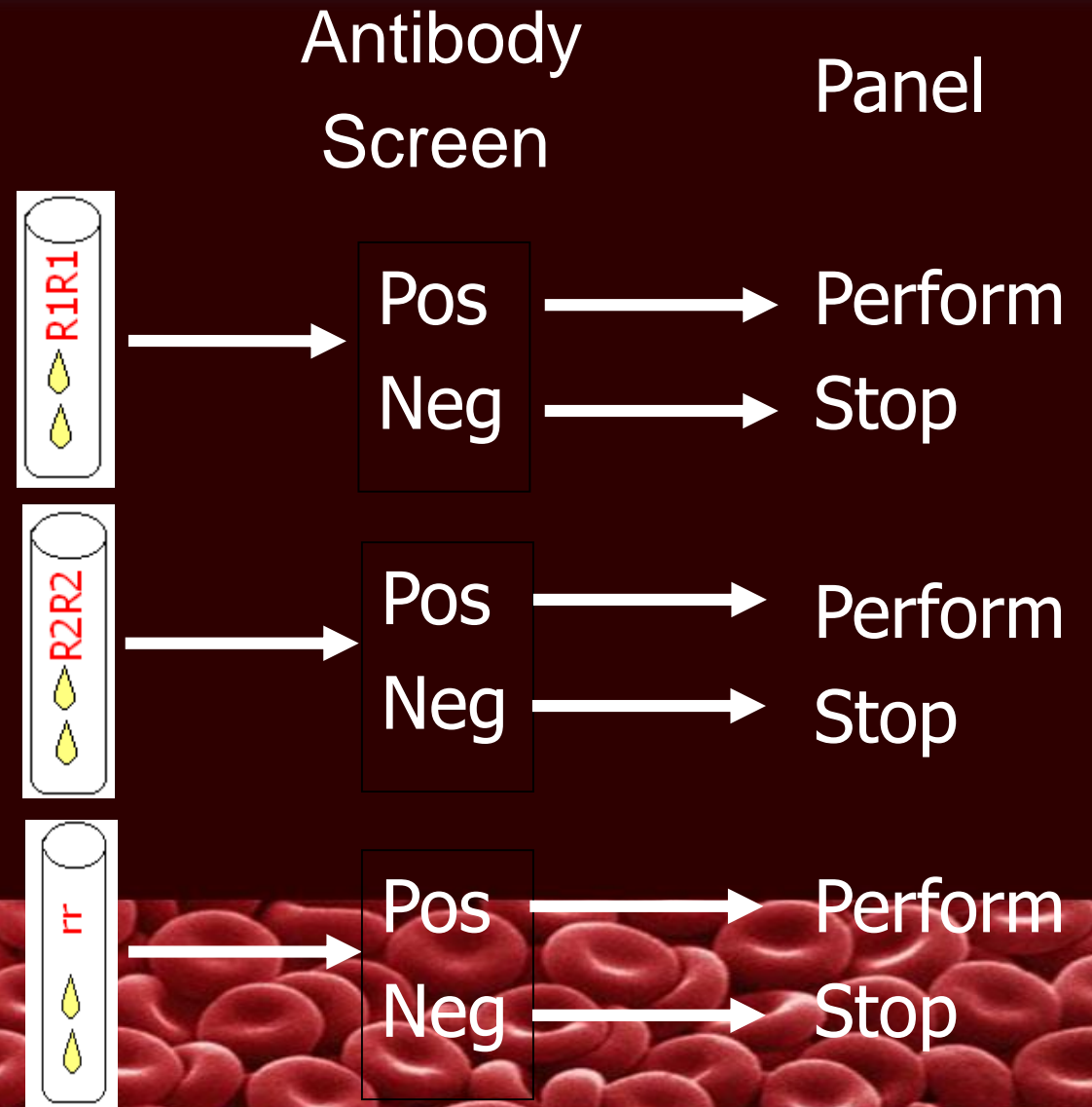
ZZAP ↓ thiol enzyme





# WAIHA Alloadsorptions

- Test adsorbed sera with screen cells
  - If negative, stop
  - If positive, perform panel and identify alloantibody(-ies) present



# WAIHA Alloabsorptions

<b>Phenotype after ZZAP treatment</b>	<b>Possible alloantibodies present in adsorbed serum</b>
R1R1, Jk(a+b-)	Anti-c, -E, -K, -Fya, -Fyb, -Jkb, -M, -N, -S, -s
R2R2, Jk(a-b+)	Anti-C, -e, -K, -Fya, -Fyb, -Jka, -M, -N, -S, -s
rr, Jk(a-b+)	Anti-D, -C, -E, -K, -Fya, -Fyb, -Jka, -M, -N, -S, -s





# WAIHA Alloadsorptions

Phenotype after ZZAP treatment	Possible alloantibodies present in adsorbed serum
R1R1, Jk(a+b-)	Anti-c, -E, -K, -Fya, -Fyb, -Jkb, -M, -N, -S, -s
R2R2, Jk(a-b+)	Anti-C, -e, -K, -Fya, -Fyb, -Jka, -M, -N, -S, -s
rr, Jk(a-b+)	Anti-D, -C, -E, -K, -Fya, -Fyb, -Jka, -M, -N, -S, -s

R1R1	AHG		R2R2	AHG		rr	AHG
I	0		I	0		I	2+
II	0		II	0		II	2+
III	0		III	0		III	0
AC	0		AC	0		AC	0



# WAIHA Alloadsorptions

Phenotype after ZZAP treatment	Possible alloantibodies present in adsorbed serum
R1R1, Jk(a+b-)	Anti-c, -E, -K, -Fya, -Fyb, -Jkb, -M, -N, -S, -s
R2R2, Jk(a-b+)	Anti-C, -e, -K, -Fya, -Fyb, -Jka, -M, -N, -S, -s
rr, Jk(a-b+)	Anti-D, -C, -E, -K, -Fya, -Fyb, -Jka, -M, -N, -S, -s

R1R1	AHG	R2R2	AHG	rr	AHG
I	0	I	1+	I	1+
II	0	II	0	II	0
III	0	III	0	III	0
AC	0	AC	0	AC	0





# WAIHA Alloadsorptions

Phenotype after ZZAP treatment	Possible alloantibodies present in adsorbed serum
R1R1, Jk(a+b-)	Anti-c, -E, -K, -Fya, -Fyb, -Jkb, -M, -N, -S, -s
R2R2, Jk(a-b+)	Anti-C, -e, -K, -Fya, -Fyb, -Jka, -M, -N, -S, -s
rr, Jk(a-b+)	Anti-D, -C, -E, -K, -Fya, -Fyb, -Jka, -M, -N, -S, -s

R1R1	AHG	R2R2	AHG	rr	AHG
I	0	I	0	I	0
II	0	II	0	II	0
III	2+	III	2+	III	2+
AC	0	AC	0	AC	0



# WAIHA Alloadsorptions

Phenotype after ZZAP treatment	Possible alloantibodies present in adsorbed serum
R1R1, Jk(a+b-)	Anti-c, -E, -K, -Fya, -Fyb, -Jkb, -M, -N, -S, -s
R2R2, Jk(a-b+)	Anti-C, -e, -K, -Fya, -Fyb, -Jka, -M, -N, -S, -s
rr, Jk(a-b+)	Anti-D, -C, -E, -K, -Fya, -Fyb, -Jka, -M, -N, -S, -s

R1R1	AHG	R2R2	AHG	rr	AHG
I	0	I	0	I	0
II	0	II	0	II	0
III	2+	III	2+	III	2+
AC	0	AC	0	AC	0





# WAIHA Transfusions

- WAA → all crossmatches incompatible
  - Determine alloantibodies present
  - Transfuse antigen-negative units
- WAAs demonstrating specificity (i.e. autoanti-e)
  - Antigen-negative units may survive longer
  - Sometimes not feasible (e.g. RhD negative patient)



# WAIHA Transfusions

“Transfused RBCs will generally survive only as long as the patient’s own cells. In addition, transfusion may stimulate the formation of other allo- or autoantibodies. Judicious RBC transfusion, following consultation between the attending MD and the transfusion service MD, is the most prudent course of action. Least-incompatible ABO/Rh compatible donor RBCs may be transfused if clinically indicated.”





# WAIHA Treatment

- Immunosuppressants or corticosteroids
  - 80-90% idiopathic AIHA respond
  - 2/3 achieve complete remission
- Secondary AIHA
  - Primary disease needs addressing
- RBC transfusion should **ONLY** be supportive!



# Conclusion

- WAIHA cause problems for transfusion services
- WAA can mask alloantibodies, making selection of units to transfuse difficult
- WAA must be adsorbed out to detect possible alloantibodies
- Transfusion of patients with WAIHA should only be done as adjunct to other therapies



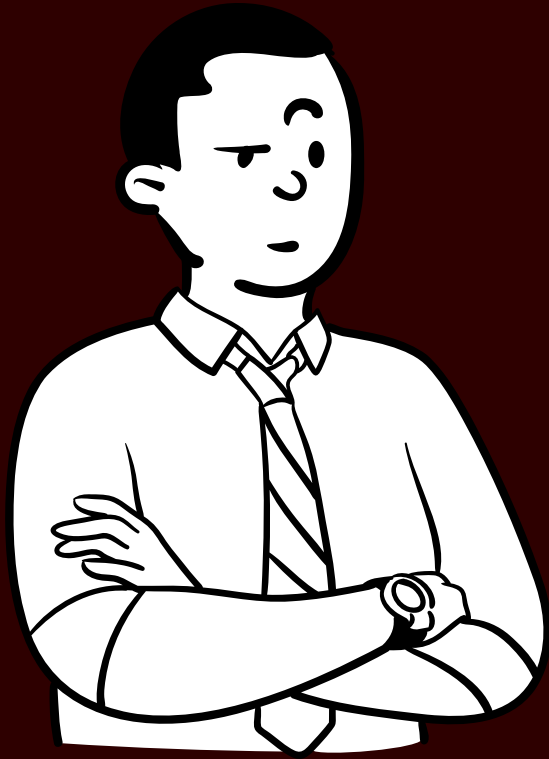


# References

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# WAIHA



Questions?

