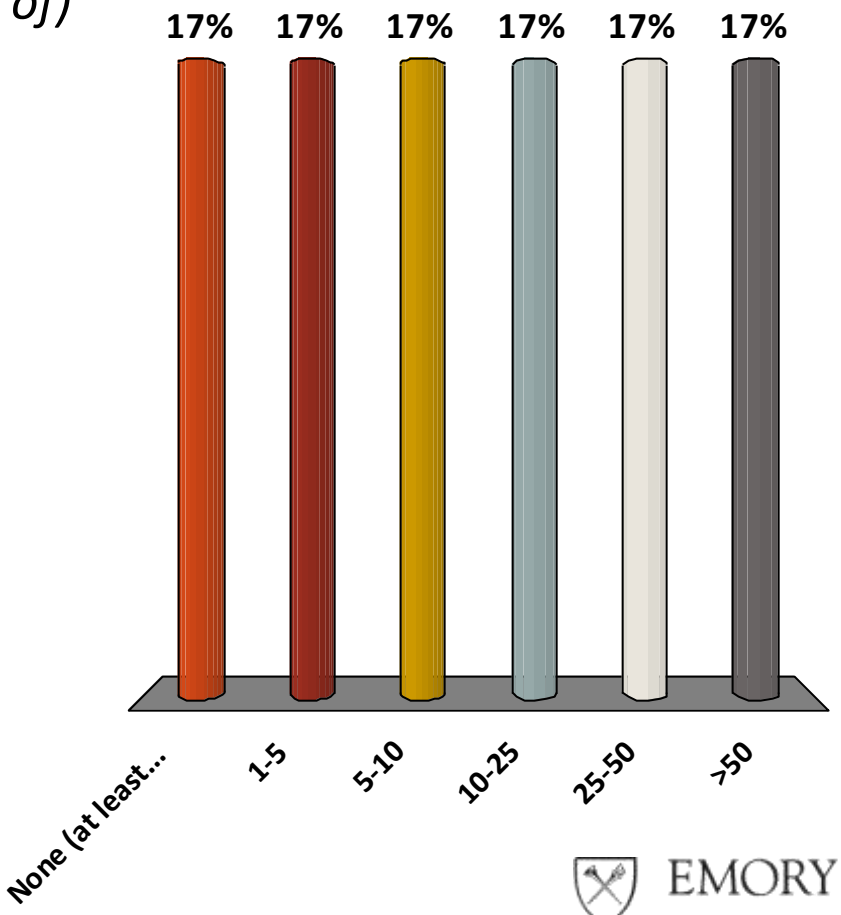
Transfusion Practices and Creation of a Registry for Patients with Sickle Cell Disease within the Atlanta Sickle Cell Consortium

Annie Winkler MD
Assistant Professor, Emory University Department of Pathology and Laboratory Medicine
Medical Director, Grady Health System Transfusion Service
Assistant Medical Director, Emory Special Coagulation Laboratory

SEABB Annual Meeting
March 6, 2013

My BB/transfusion service provides care to how many SCD patients per month?

1. None *(at least that your informed of)*
2. 1-5
3. 5-10
4. 10-25
5. 25-50
6. >50



EMORY
tm & coag

Case Presentation

- 14 year old female with sickle cell disease (SCD) transferred from Hughes Spalding to Egleston for unresponsive left arm pain due to a moderate sized joint effusion
  - Of note, she had been admitted on 3 separate occasions in the last 2 weeks for vaso-occlusive crisis and possible acute chest syndrome
- The patient was also being evaluated for possible inflammatory bowel disease after findings of abdominal lymphadenopathy on CT and a positive stool guaiac were noted on a recent admission
- GI was planning upper and lower endoscopies
  - Procedures to take place at Egleston, type and screen and RBCs ordered
    - Hemoglobin 8.8 g/dl

Case Presentation: Transfusion History

- Phenotype

C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s
0	0	+	+	+	+	0	0	+	0	0	0	+	+	+	0	+

- Prior to 2/27/2012, the patient had negative antibody screens at Grady/Hughes Spalding and she was receiving C-E- and HbS negative units (total of 5 RBCs from 4/2011-11/2011)

- On 2/27/2012, Egleston identified and ARC confirmed an e-like antibody which was not able to be classified as auto or alloimmune
  - Suspicion for an anti-hr^B
  - Referred for molecular testing
    - Human erythrocyte antigen (HEA)
    - *RHCE* genotyping

Case Presentation: Transfusion History

- With this information unbeknownst to Grady Blood Bank, the patient was admitted to Hughes Spalding, and type and screen request for transfusion of one unit pRBCs was sent
  - Because of the e-like antibody, Grady issued units that were C- **e** **-**Fy^a- Jk^b- and HbS negative
- 6/27/2012: new alloantibody identified

Case Presentation: Molecular Results

- Patient was confirmed to be a partial c, partial e, and hr^B negative

TESTING REQUESTED: Genotype for *RHe* variants

			RESULT	INTERPRETATION
RHD gene	PCR	RHD Exon 4	present	D+/D+
		RHD Exon 7	present	
		Pseudogene	absent	
	PCR	Hybrid Rhesus box	absent	
RHCE gene	PCR, HEA	C	absent	cc
		c	present	
			Genotype	
RHCE Exon 5	HEA	676G>C (A226P)	GG	ee
			Genotype	
RHCE Exon 1	RFLP	48G>C (W16C)	GC	W16C
RHCE Exon 2	RFLP	254C>G (A85G)	CC	A85
RHCE Exon 5	HEA	733C>G (L245V)	GG	245V
RHCE Exon 7	HEA	1006G>T (G336C)	GG	G336
	RFLP	1025C>T (T342I)	CC	T342
RHCE SEQUENCING:	gDNA seq	Exon 5	733G/G	245V

***RH* Genotype:** $R_0^{cc\ variant}$ / $R_0^{ce\ variant}$; specifically *Dce(733G)/Dce(48C, 733G)*

Predicted phenotype (based on these results and HEA results):
D+, C-E-, partial c+, partial e+, VS+V+, and hr^B-/+^{w}

- New unit requirements: C- E- hr^B- Fy^a- Jk^b- and HbS negative

EMORY tm & coag

Case Presentation Learning Points

- Not only is alloimmunization complex, but also is the infrastructure of our health care systems
  - Leads to gaps in communication which are potentially harmful for patient care

- Importance of red cell molecular testing for multiply transfused, alloimmunized SCD patients

Objectives

- Review alloimmunization rates and pathobiology, unit selection and phenotype matching for transfusion of patients with sickle cell disease
- Discuss the role of red blood cell genotyping for patients with sickle cell disease
- Introduce the creation and use of a registry for patients with sickle cell disease within the Atlanta Sickle Cell Consortium

Alloimmunization Rates and Pathobiology

Alloimmunization in SCD

The New England
Journal of Medicine

Volume 322 JUNE 7, 1990 Number 23

ALLOIMMUNIZATION IN SICKLE CELL ANEMIA AND TRANSFUSION OF RACIALLY UNMATCHED BLOOD

ELLIOTT P. VICHINSKY, M.D., ANN EARLES, P.N.P., ROBERT A. JOHNSON, M.D., M. SILVIJA HOAG, M.D., AMBER WILLIAMS, M.T., AND BERTRAM LUBIN, M.D.

- Much of what we know about the natural history of alloimmunization in SCD originated from this 158 patient cohort of patients from Children's Hospital Oakland (1978-1985)

Table 1. Frequency of Alloimmunization among Patients with Sickle Cell Anemia and Patients with Other Forms of Chronic Anemia.

Diagnosis	Total No. of Patients	Patients Given Transfusions	Patients with Alloantibodies
Sickle cell anemia	158	107	32 (30%)
Children (mean age, 10 yr; range, 1–17)	85	42	10 (24%)
Adults (mean age, 24 yr; range, 18–41)	73	65	22 (34%)
Chronic anemia (children: mean age, 11 yr; range, 1–18)	19	19	1 (5%)*

*Value is significantly lower than that for patients with sickle cell anemia ($P<0.001$).

Table 2. Relation of the Number of Transfusions to the Appearance of Red-Cell Antibodies in Sickle Cell Anemia.

Diagnosis	No. of Patients	No. of Transfusions	
		Average	Range
Chronic anemia	19	131	3–600
Sickle cell anemia			
No alloantibodies	75	13	1–45
Alloantibodies	32	23	3–46
Appearance of first antibody		12*	1–31

*The average number of transfusions received by the time the first red-cell antibody appeared.

Table 3. Distribution of the 68 Red-Cell Alloantibodies in 107 Patients Receiving Transfusions for Sickle Cell Anemia.

Antibody	No. (%)
K	18 (26)
E	16 (24)
C	11 (16)
Jk^b	7 (10)
Fy^a	4 (6)
M	3 (4)
Le^a	3 (4)
S	2 (3)
Fy^b	2 (3)
e	1 (2)
Jk^a	1 (2)

Vichinsky EP. *N Engl J Med* 1990, 322: 1617-1621

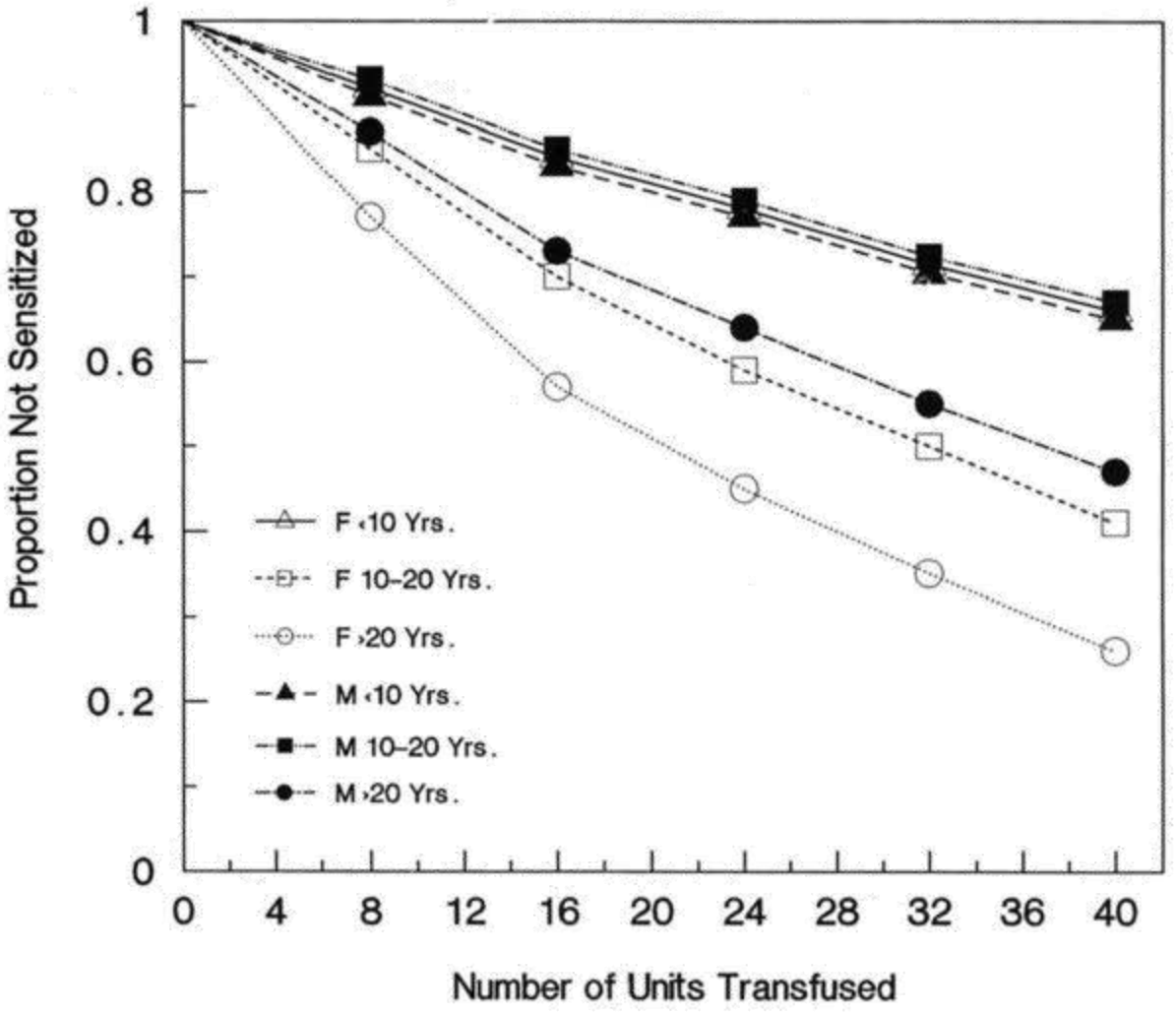
EMORY tm & coag

Alloimmunization in SCD

- Vichinsky *et al* concluded that the risk of alloimmunization is greater in patients receiving transfusions for sickle cell anemia than in patients receiving transfusions for other chronic disease
  - Cooperative Study of Sickle Cell Disease demonstrated a 18% alloimmunization rate from 741 patients with SCD transfused before entry
    - 18.2% of patients developed alloantibodies who had not been transfused
- Recommended that all patients with SCD who undergo transfusion be matched with donors for red cell antigens commonly associated with alloimmunization and DHTRs
  - Based upon supporting data from Ambruso *et al*
    - Prospective phenotype matching for 17 blood group antigens reduced alloimmunization 10 fold in 12 SCD patients

Vichinsky EP. *N Engl J Med* 1990, 322: 1617-1621
Rosse WF. Blood 1990, 76: 1431-1437
Ambruso DR. *Transfusion* 1987, 27: 94-98

Alloimmunization in SCD



Rosse WF. Blood 1990, 76: 1431-1437

EMORY
tm & coag

Alloimmunization in SCD: 30 Years Later

- As part of the PROACTIVE Feasibility Study, transfusion histories were obtained from 237 pediatric (51.5%) and adult (48.5%) patients at 26 centers participating in the Sickle Cell Disease Clinical Research Network
  - Of the 237 subjects, 7.1% had alloimmunization cited as a reason for exclusion
    - >75% of patients had been transfused

TABLE 1. Transfusion history at enrollment by age group

Number of prior RBC transfusions	Age group (years) 2-9 (n = 44) Number of patients with alloantibody (n = 3)	2-9 (n = 44) Total number of patients (%)	10-17 (n = 78) Number of patients with alloantibody (n = 11)	10-17 (n = 78) Total number of patients (%)	18-35 (n = 92) Number of patients with alloantibody (n = 15)	18-35 (n = 92) Total number of patients (%)	36+ (n = 22) Number of patients with alloantibody (n = 5)	36+ (n = 22) Total number of patients (%)	Total (n = 236) Number of patients with alloantibody (n = 34)	Total (n = 236) Total number of patients (%)
None	0	14 (31.8)	0	17 (21.8)	1	22 (23.9)	0	4 (18.2)	1	57 (24.2)
1-5	2	20 (45.5)	7	36 (46.2)	3	27 (29.3)	1	5 (22.7)	13	88 (37.3)
5-10	0	5 (11.4)	1	13 (16.7)	4	22 (23.9)	1	3 (13.6)	6	43 (18.2)
>10	1	5 (11.4)	3	12 (15.4)	7	21 (22.8)	3	10 (45.5)	14	48 (20.3)

  - The alloimmunization rates of heavily transfused subjects was no different in either adult or pediatric patients

Miller ST. *Transfusion* 2012, *epub ahead of print*

EMORY tm & coag

Alloimmunization in SCD: 30 Years Later

- When participating centers were surveyed about blood bank and transfusion practices, the following was reported
  - 9 of 11 (82%) centers performed RBC phenotyping
  - 19 of 23 (83%) centers antigen matched beyond ABO/Rh and known antibodies
    - 3 only if one or more antibodies were known
    - 1 center on a “case by case basis”
  - Interestingly, there was no difference in antibody prevalence between the 4 centers who did not routinely provide antigen matched blood (13.7%) and those that did (14.8%)
    - No additional difference between C, E, K versus extended matching

Alloimmunization with Chronic Transfusion

- Wahl *et al* recently reported alloimmunization rates in 45 pediatric patients on chronic transfusion therapy at Children's Hospital and Research Center Oakland
  - 22 erythrocytapheresis (ECP)/RBC exchange
  - 23 simple transfusion

TABLE 5. Antibody formation rates per 100 units transfused

Transfusion regimen	Patients with any new antibody, n (%)	Total units during program	Total number of new antibodies formed (new allo)	Total antibodies (auto plus allo) per 100 transfused units	Allo antibodies per 100 transfused units
ECP, n = 22	3 (13)	7447	3 (1 allo)	0.040	0.013
Simple transfusion, n = 23	4 (18)	3502	6 (5 allo)	0.171	0.143
p value				0.04	0.03

  - Similar to 2 recent reports, alloimmunization rates were lower in the ECP group

RBC Alloimmunization Pathobiology

- Alloimmunization to erythrocytes involves multiple steps including:
  - RBC antigen recognition, processing, and presentation by HLA class II to T-cell receptors
  - Activation of CD4 helper T cells
  - Interaction of T and B cells
  - B-cell differentiation into plasma cells
- Murine and human studies have shown that the process can be modulated at each step through both acquired and genetic factors; however, the clinical relevance of these factors in SCD have not been completely elucidated

RBC Alloimmunization Pathobiology: RBC Antigen Factors

- Antigenic differences between donor and recipient RBCs are requisite for the initial trigger for alloimmunization
  - In the US, alloimmunization rates for patients with SCD range from 20-50% in comparison to 6.1% and 2.6% in Uganda and Jamaica, respectively

- Antigenic differences between donors and SCD patients have three levels of complexity
  1. Prevalence of some common but highly immunogenic antigens differs substantially between donors and transfusion recipients
  2. Transfusion of Rh compatible units does not entirely prevent the risk of alloimmunization because of the prevalence of Rh variants found in persons of African descent
  3. High Incidence Antigens

Difference In Minor Antigen Prevalence between Racially Different Pairs

Antigen	% in white donors	% in black recipients
D	85	92
C	**68**	**27**
E	**29**	**20**
c	80	96
e	98	98
K	**9**	**2**
Fy^a	**66**	**10**
Fy^b	83	23
Jk^a	77	92
Jk^b	**74**	**49**
S	**51**	**31**
s	89	93

Difference In Minor Antigen Prevalence between Racially Different Pairs

Antigen	% in white donors	% in black recipients
D	85	92
C	**68**	**27**
E	**29**	**20**
c	80	96
e	98	98
K	**9**	**2**
Fy^a	**66**	**10**
Fy^b	83	23
Jk^a	77	92
Jk^b	**74**	**49**
S	**51**	**31**
s	89	93

Table 4. Red-Cell Phenotypes of Patients with Sickle Cell Anemia and Local Blood-Bank Donors.

Phenotype	Patients (N = 158)	Donors (N = 200)	P Value*
	percent with phenotype		
c	99	81	NS
P1	99	79	NS
e	98	98	NS
s	95	94	NS
Jk^a	91	77	NS
N	77	74	NS
M	69	80	<0.01
Le^b	45	72	<0.001
Jk^b†	39	72	<0.001
C†	28	68	<0.001
S	26	55	<0.001
E†	24	35	<0.01
Le^a	21	22	NS
Fy^a†	15	67	<0.001
Fy^b	11	82	<0.001
K†	2	9	<0.001

*Significance of the decrease in antigen frequency in the patients as compared with the donors. NS denotes not significant.

†Eighty-two percent (56 of 68) of the alloantibodies in these patients were directed against these antigens.

EMORY tm & coag

Vichinsky EP. *N Engl J Med* 1990, 322: 1617-1621

Additional Antigen Difference Complexity

- Rh variant antigens account for the second level of antigenic complexity between donor and patient RBCs

Antigen	% in white donors	% in black recipients
Partial D among D+	1	7
Partial C among C+	0	30
Partial e among e+	0	2

  - Partial alleles are most often not recognized until an alloantibody has formed due to the limitations of serologic phenotyping

- The third level of antigenic complexity between SCD patients and donor RBCs arises when the recipient lacks a high incidence antigen

RBC Alloimmunization Pathobiology: Individual Specific Factors

- Similar to platelet antigen alloimmunization, the HLA class II genotype of the patient has been shown to be a predictor of a patient's responder status
  - For example, HLA-DRB1*1503 has been associated with increased risk of alloimmunization while HLA-DRB1*0901 appears to be protective
- Data from mouse models also demonstrates that T regulatory cells (Treg) inhibit the magnitude and frequency of alloimmunization
  - Patients with weaker Treg activity are unable to suppress antibody production compared with non-responders
    - Demonstrated in a small cohort of chronically transfused SCD patients from Uganda
- May also be other immune factors such as T-cell subgroups, increased levels of circulating cytokines (TGF-β and IL-6) that play a role in RBC alloimmunization

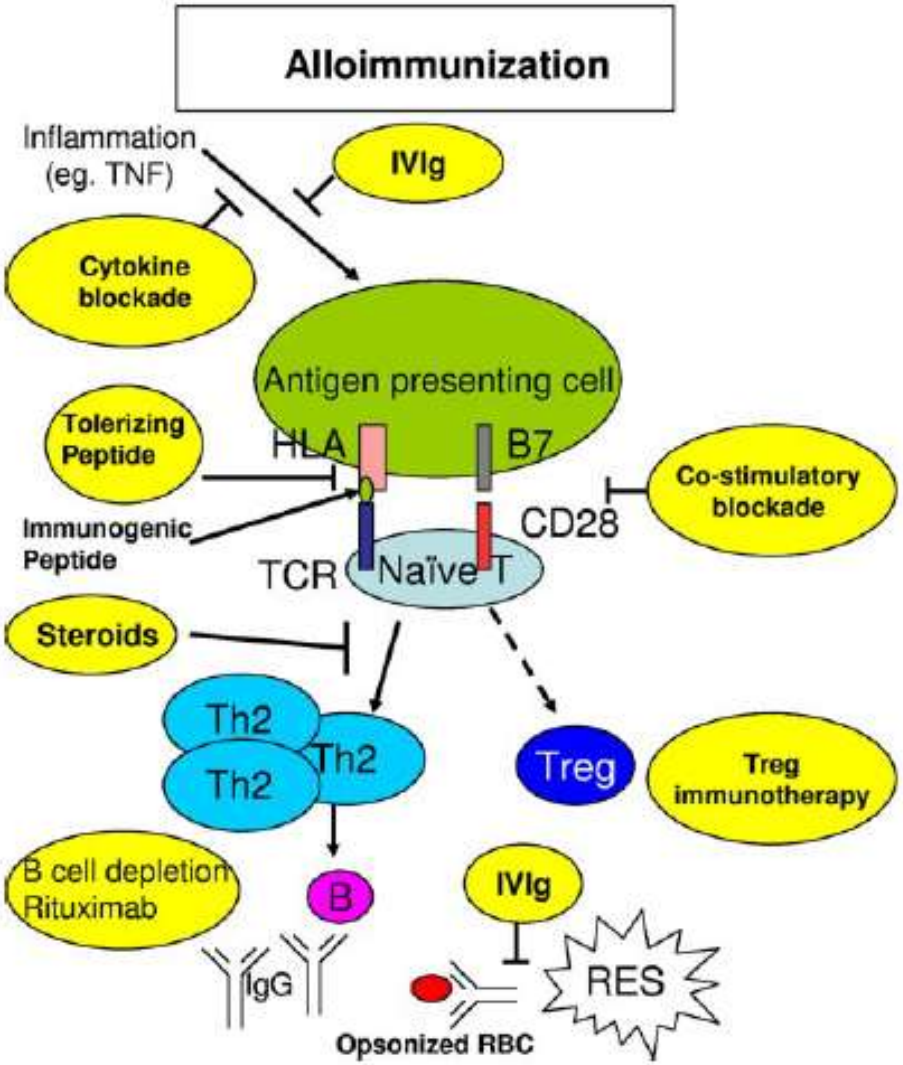
RBC Alloimmunization Pathobiology: SCD Specific Factors

- One of the main features of SCD is a chronic inflammatory state
  - Evidenced by increased levels of CRP and inflammatory cytokines (IL1, IL-6, INF-γ), as well as increased WBC counts
- In mouse models, certain pro-inflammatory stimuli enhance alloimmunization
  - Hendrickson *et al* demonstrated RBC consumption by splenic and hepatic dendritic cells which are potent inducers of alloimmunization
- Published literature also has demonstrated that a previous febrile non-hemolytic transfusion reaction was associated with an increased risk of RBC alloimmunization
  - Although findings have not been specifically confirmed in SCD

RBC Alloimmunization Pathobiology: SCD Specific Factors

- Other unknown SCD factors
  - It is unknown whether alloimmunization rates differ depending on the presence or absence of clinical complications of SCD, frequency of transfusion, or age at first transfusion
    - For example, is vaso-occlusive crisis, which is associated with increased inflammatory markers, have an altered alloimmunization potential?
    - Or are children who are chronically transfused have less inflammation that may be responsible for lower rates of alloimmunization?
    - Or does starting chronic transfusion at a younger age induce immune tolerance and therefore lower rates of alloimmunization?
  - Identification of genetic markers predictive of immunization
    - It has been shown that a polymorphism in the immunoregulatory TRIM21 gene, which is in close proximity to the β-globin locus, is associated with an increased rate of SCD alloimmunization especially in early childhood

RBC Alloimmunization Pathobiology: Hypothetical Schema



Yazdanbakhsh K. *Blood* 2012, 120: 528-537

EMORY tm & coag

RBC Phenotype Matching Protocols and Outcomes

Prospective Antigen Matching Protocols

- Prospective phenotype matching started in single centers as early as 1987, but were not widely adopted until the early 2000s following publication of the STOP trial
  - Stroke Prevention Trial in Sickle Cell Anemia was a multicenter randomized controlled trial comparing stroke risk in patients randomized to transfusion (n=63) versus standard arm (n=67)
    - Patients were required to receive C-E-K- matched units

TABLE 3. New antibody development during the STOP study

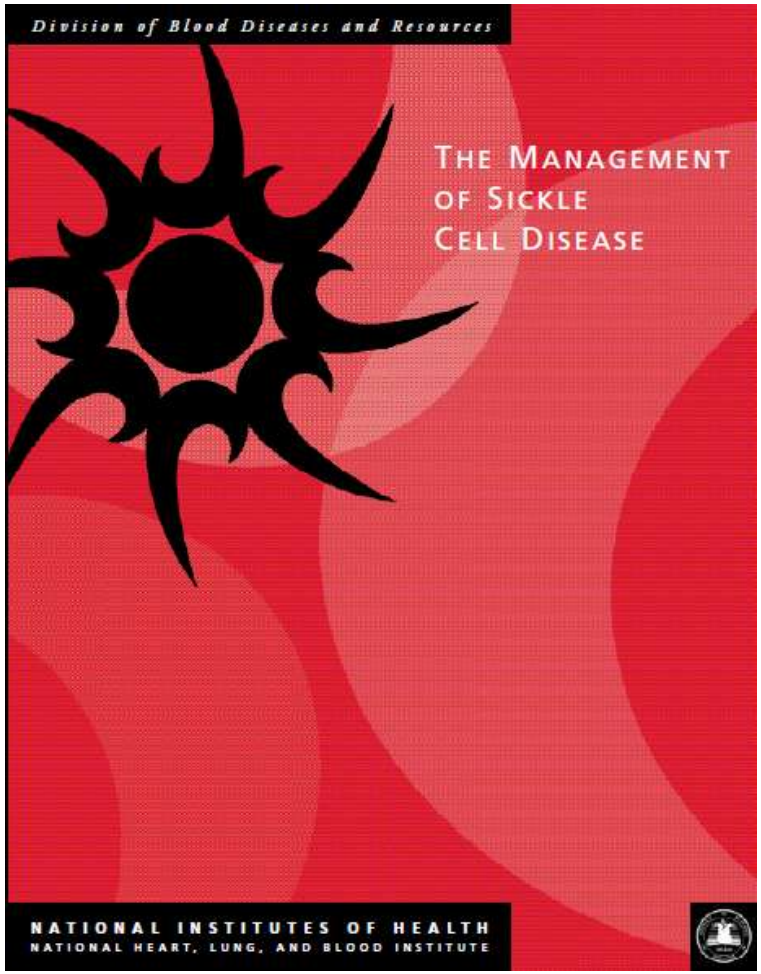
Type of reaction	Number of patients	Percentage of total	Antibody specificity in each patient
Clinically significant alloantibody	5	8%	1. Anti-E 2. Anti-E 3. Anti-K 4. Anti-K 5. Anti-Fy^a, -Le^a, -Le^b, -S
Clinically insignificant alloantibody	2	3%	1. Anti-Lu^a 2. Anti-Le^a
Warm autoantibody*	3	5%	1. Anti-e 2. Anti-e 3. Warm polyspecific
Total	10	16%	

* One warm autoantibody was excluded because it could not be verified.

- Standard Arm
  - 3%/unit
- Transfusion Arm
  - 0.5%/unit

Vichinsky EP. *Transfusion* 2001, 41: 1086-1092

Recommendations for Prospective Antigen Matching

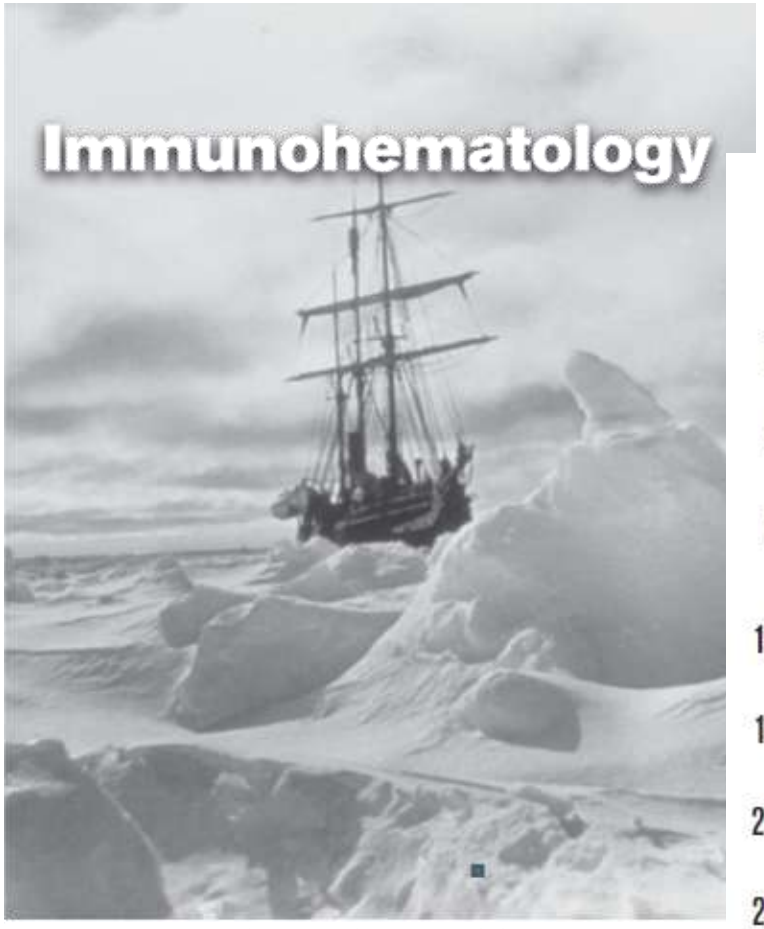


- NIH "Guidelines" were last updated in 2002 and endorsed the recommendation from the STOP trial

There are several causes of the high prevalence of alloimmunization in SCD, and phenotypic incompatibility between the donor and recipient is a major factor (7). Limited matching for E, C, and Kell antigens is usually performed, unless patients have antibodies (8).

http://www.nhlbi.nih.gov/health/prof/blood/sickle/sc_mngt.pdf

Prospective Antigen Matching Protocols



VOLUME 28, NUMBER 1, 2012

Immunohematology

Volume 28, Number 1, 2012

CONTENTS

American Red Cross

EMORY tm & coag