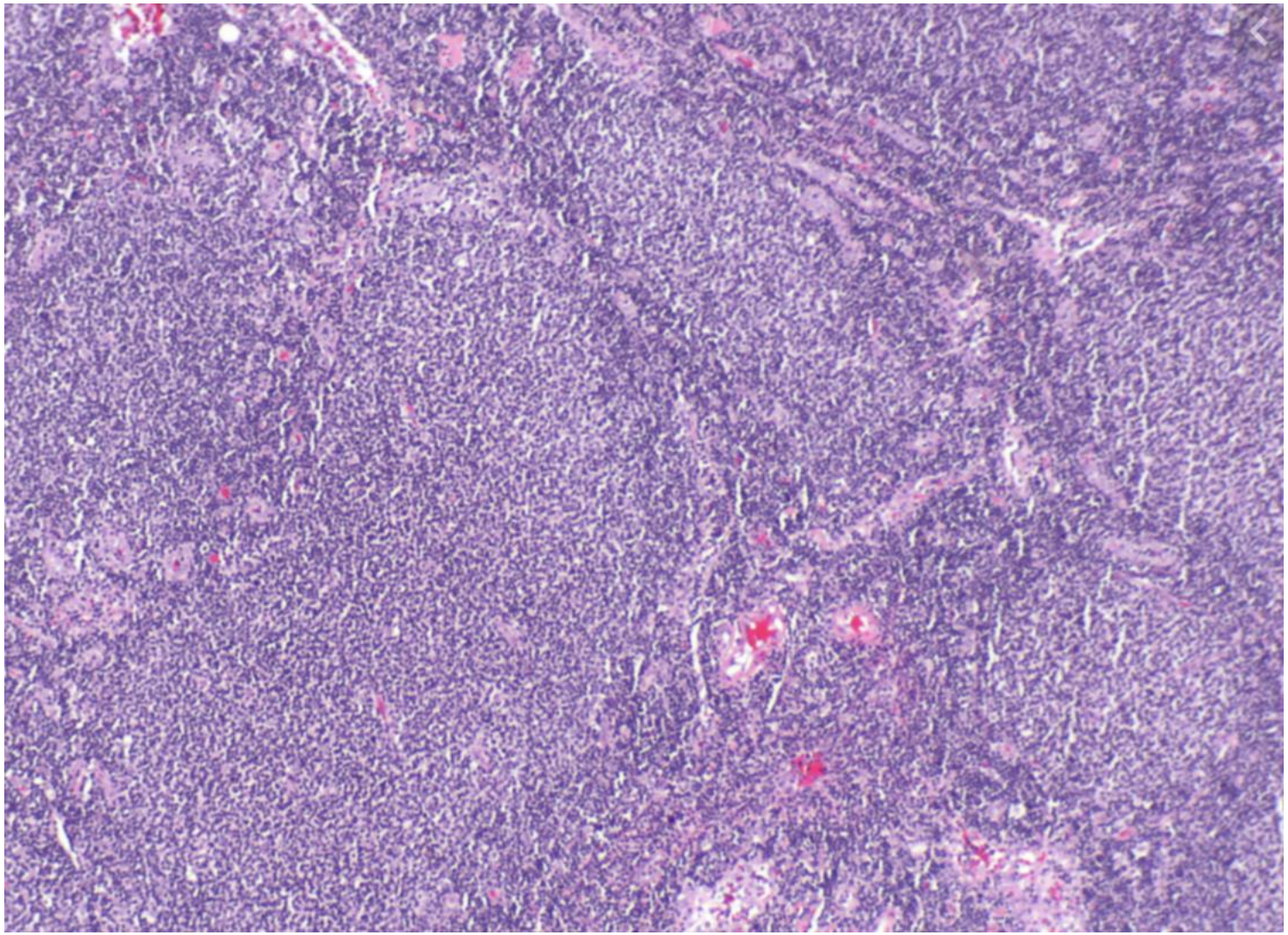
Re-examining Fundamental Concepts in Transfusion Medicine

Sean Stowell MD PhD

EMORY UNIVERSITY Center for Transfusion and Cellular Therapies

Follicular lymphoma



Why use animal models to study transfusion medicine?

- **Why do we use models?**
- **What are the advantages and limitations?**
- **What have we learned?**

Aviation

Use of models

Advantages:

- Cheaper
- Directly test hypotheses
- Access to a variety of tools

Disadvantages:

- May not recapitulate the exact situation clinically
- May lead one down the wrong path

Models

Recruiting study volunteers

$500.00 compensation

Study participants will be transfused 1 unit of ABO(H) incompatible red blood cells

Only a 50% mortality rate!*

*Based on previous anecdotal evidence – could be much better!

Sign up today!

Clinical studies

Clinical studies:

- **Determining the consequences of an intervention:**
  - **Does drug X help with condition Y?**
    - **Restrictive versus liberal transfusion thresholds**

Can be challenging when seeking to understand some mechanisms:

- **What cell(s) initiate RBC alloimmunization?**
  - **Typically employ *in vitro* studies**

Mechanistic human studies (typically *in vitro*)

Advantages:

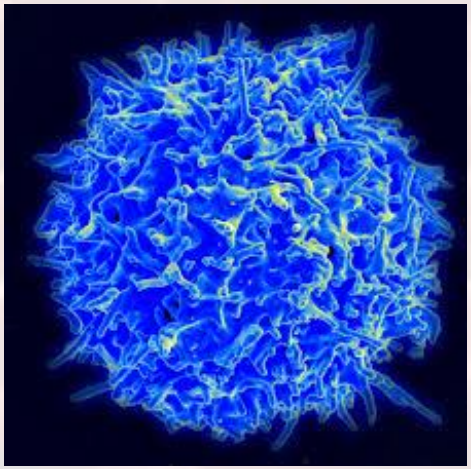
- Human (and therefore patient centered)
- Provides corollary data to mechanistic *in vivo* studies

Disadvantages:

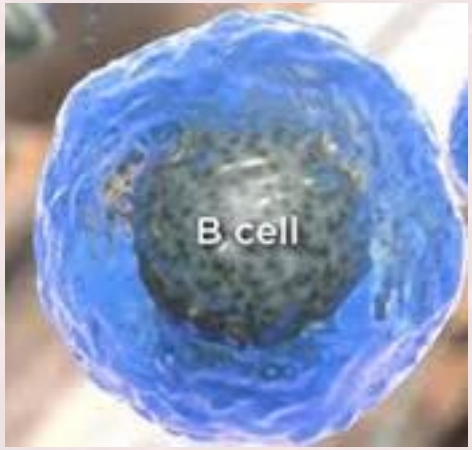
- Difficult to know if the same players are involved *in vivo*
- Primarily only assess the peripheral blood compartment
- Observations often made after immunizing event has already occurred

In vitro human studies

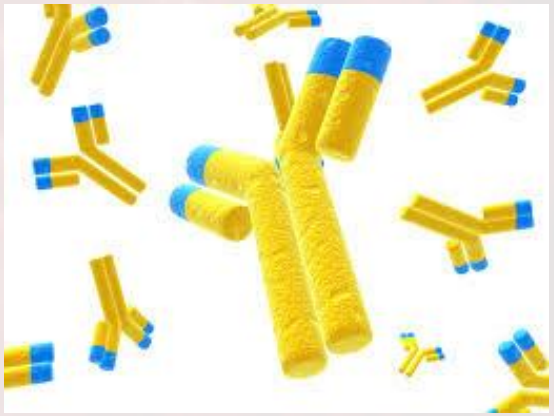
CD4 T cell



B cell



Antibody



In vitro human studies

Innate immune lectins kill bacteria expressing blood group antigen

Sean R Stowell[1,4], Connie M Arthur[1,4], Marcelo Dias-Baruffi[2], Lilian C Rodrigues[2], Jean-Philippe Gourdine[1], Jamie Heimburg-Molinaro[1], Tongzhong Ju[1], Ross J Molinaro[3], Carlos Rivera-Marrero[1], Baoyun Xia[1], David F Smith[1] & Richard D Cummings[1]

Stowell et al, Nature Medicine 2010;16:295-301.

Differential Roles of Galectin-1 and Galectin-3 in Regulating Leukocyte Viability and Cytokine Secretion[1]

Sean R. Stowell,* Yuning Qian,† Sougata Karmakar,‡ Natalia S. Koyama,§ Marcelo Dias-Baruffi,§ Hakon Leffler,† Rodger P. McEver,‡ and Richard D. Cummings[2]*

Stowell et al, Journal of Immunology 2008;283: 10109-23.

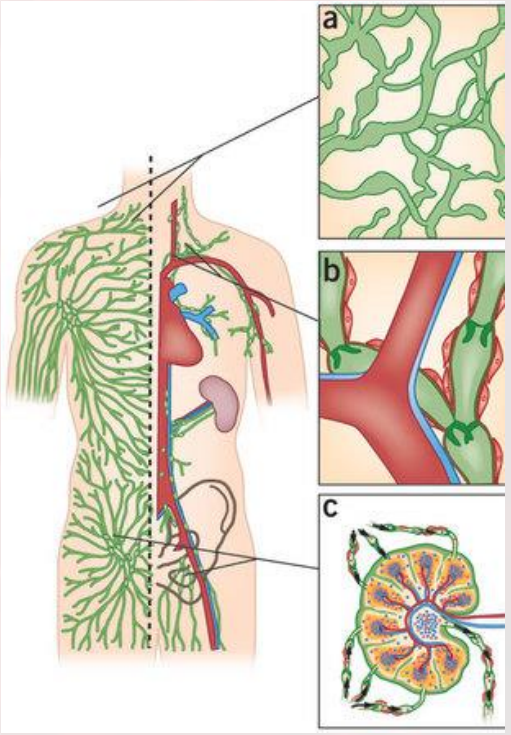
IMMUNOBIOLOGY

Human galectin-1, -2, and -4 induce surface exposure of phosphatidylserine in activated human neutrophils but not in activated T cells

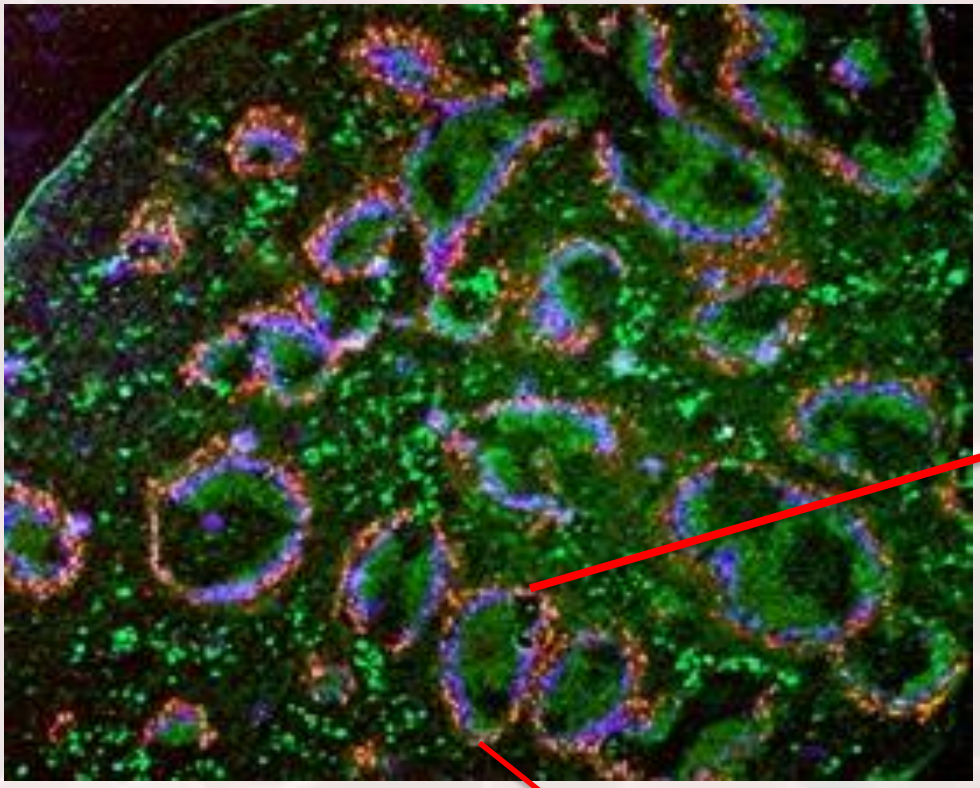
Sean R. Stowell,[1] Sougata Karmakar,[2] Caleb J. Stowell,[1] Marcelo Dias-Baruffi,[1] Rodger P. McEver,[1,2] and Richard D. Cummings[1]

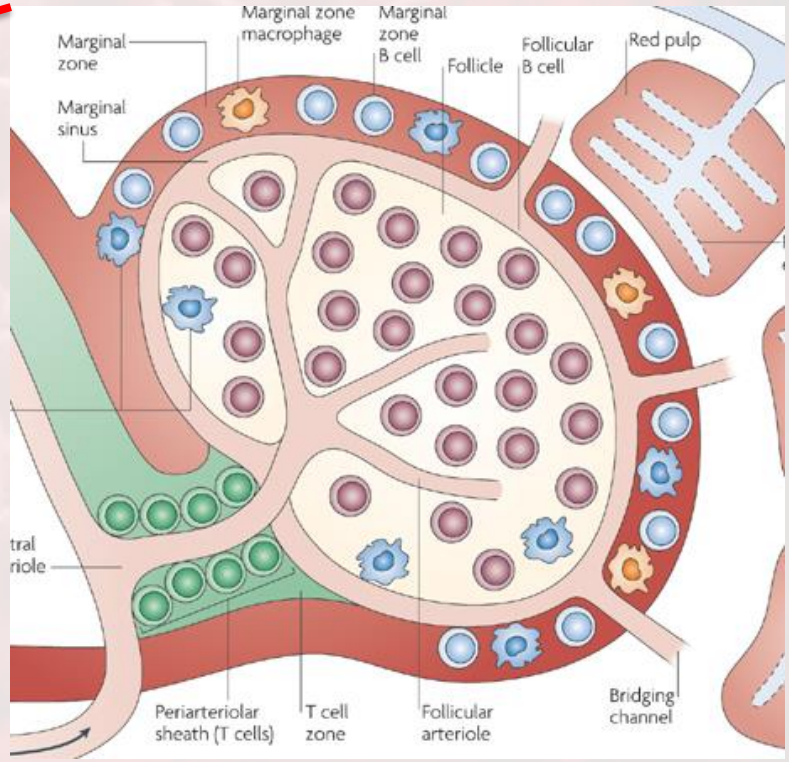
Stowell et al, Blood 2007;109:219-27.

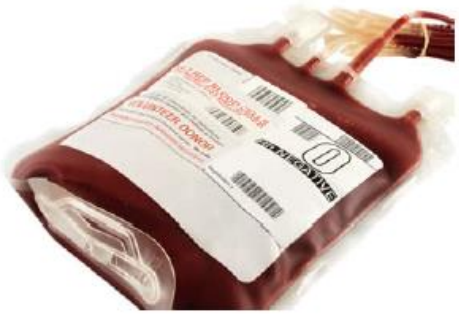
The systemic nature of the immune system



Immune architecture *in vivo*







Recruiting study volunteers

$1000.00 compensation

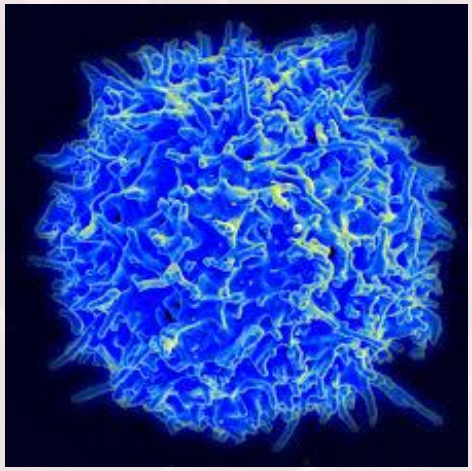
Study participants will receive 1 unit of red blood cells followed by splenectomy*

*Mild increase risk for sepsis and thrombotic complications.

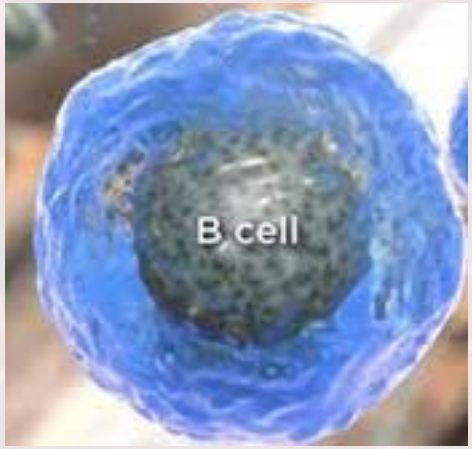
Sign up today!

In vitro human studies

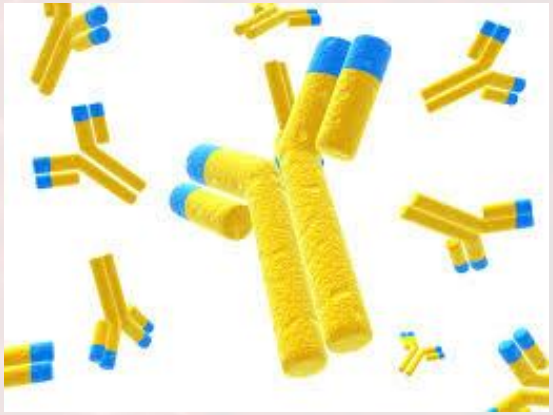
CD4 T cell



B cell



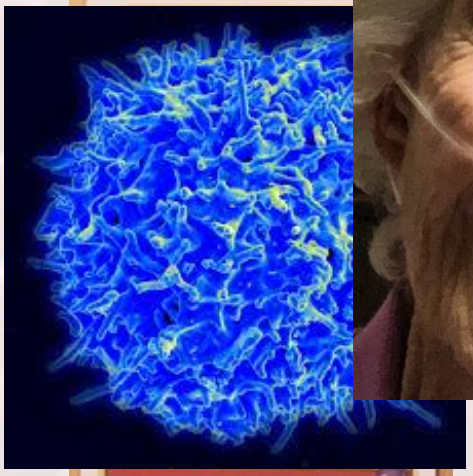
Antibody



Lymph node

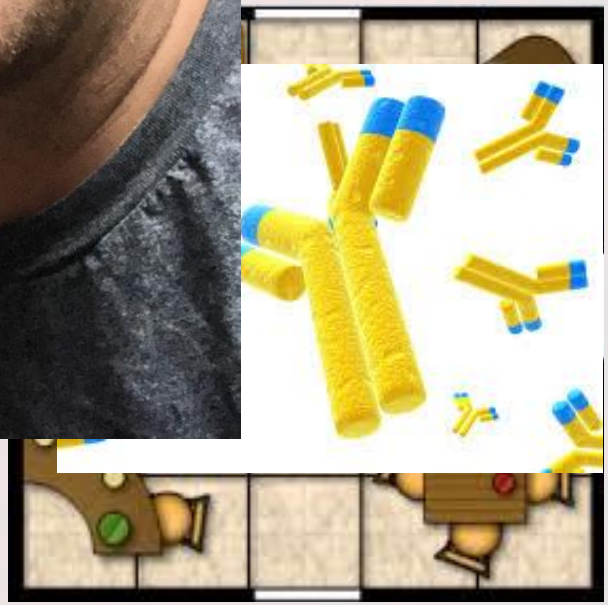
In vitro human studies

CD4 T cell



Lymph node

ntibody

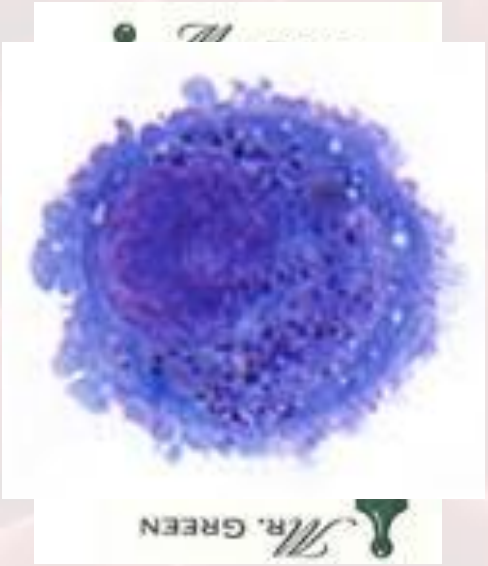


Macrophage

Dendritic cell

Neutrophil

Mast cell

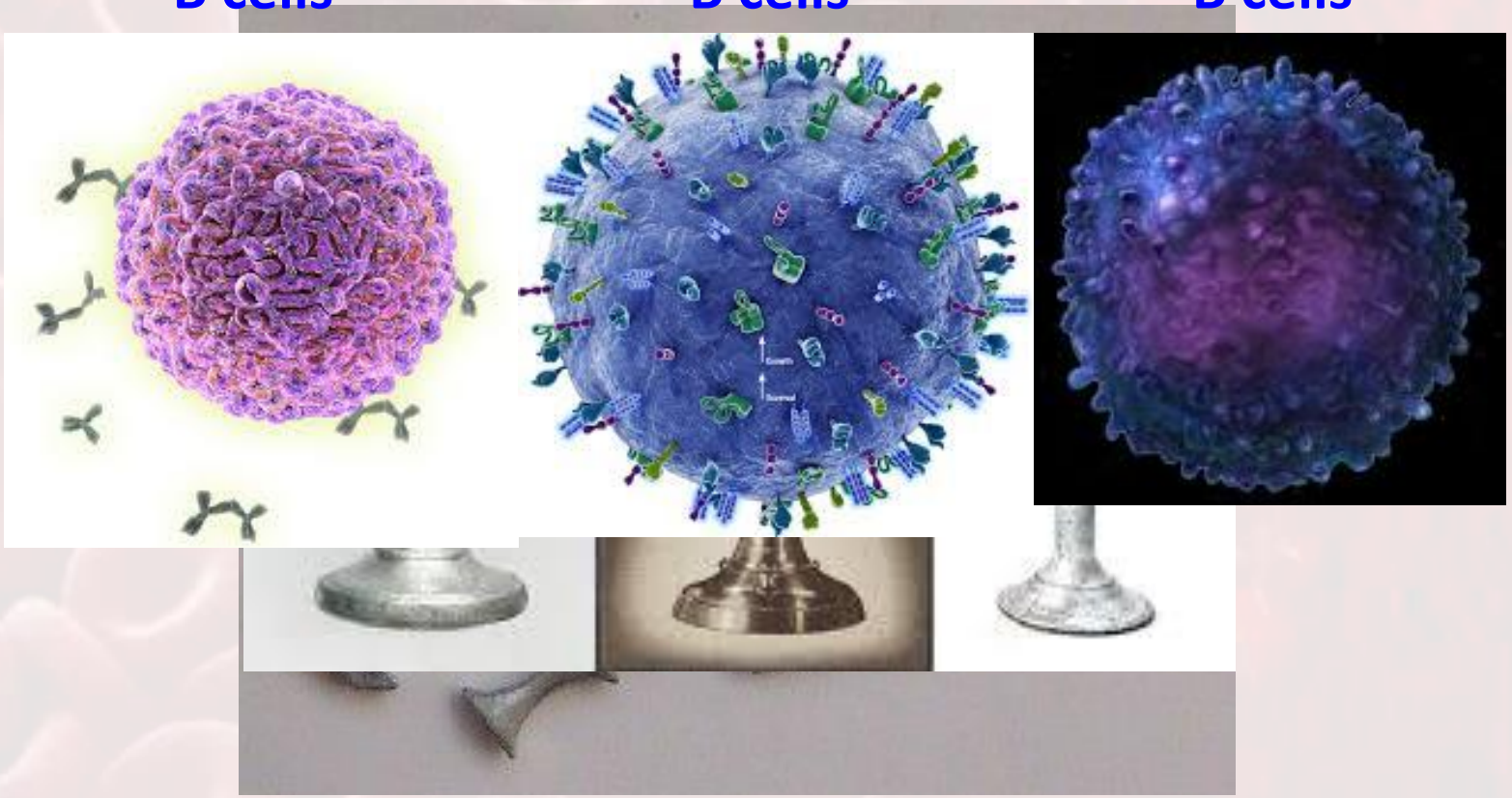


Unknown cell

Marginal Zone B cells

Follicular B cells

B1 B cells



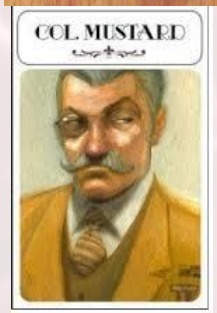
Lymph node

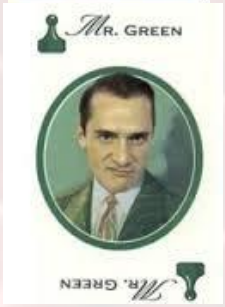
Spleen

Liver

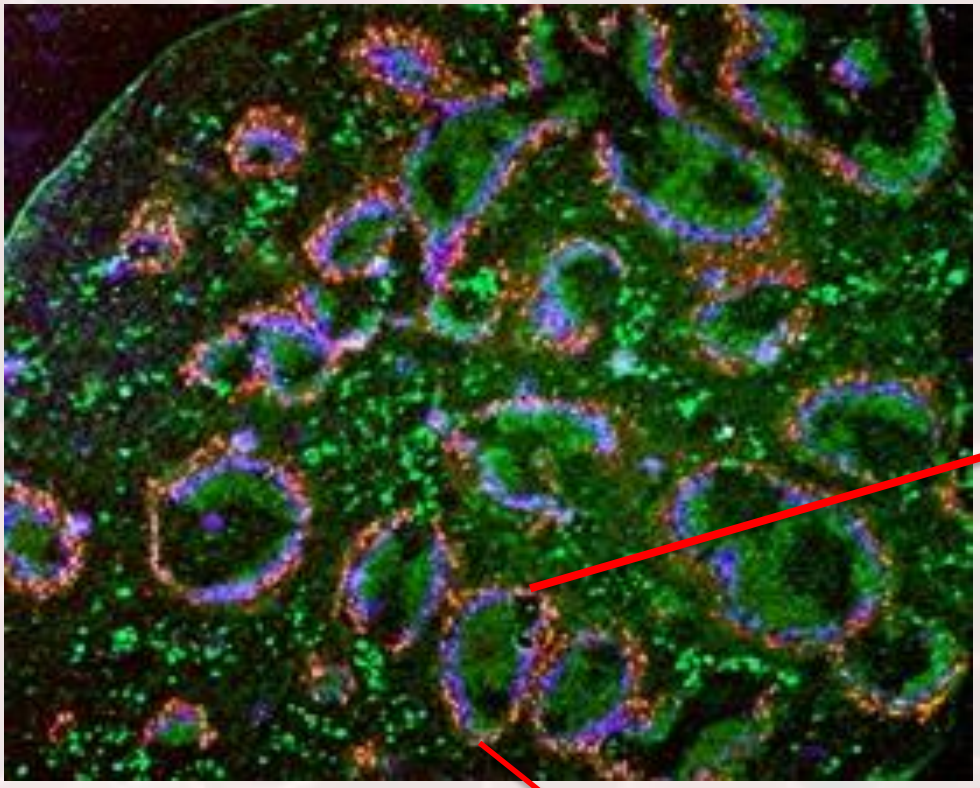
Mucosal lymph tissue

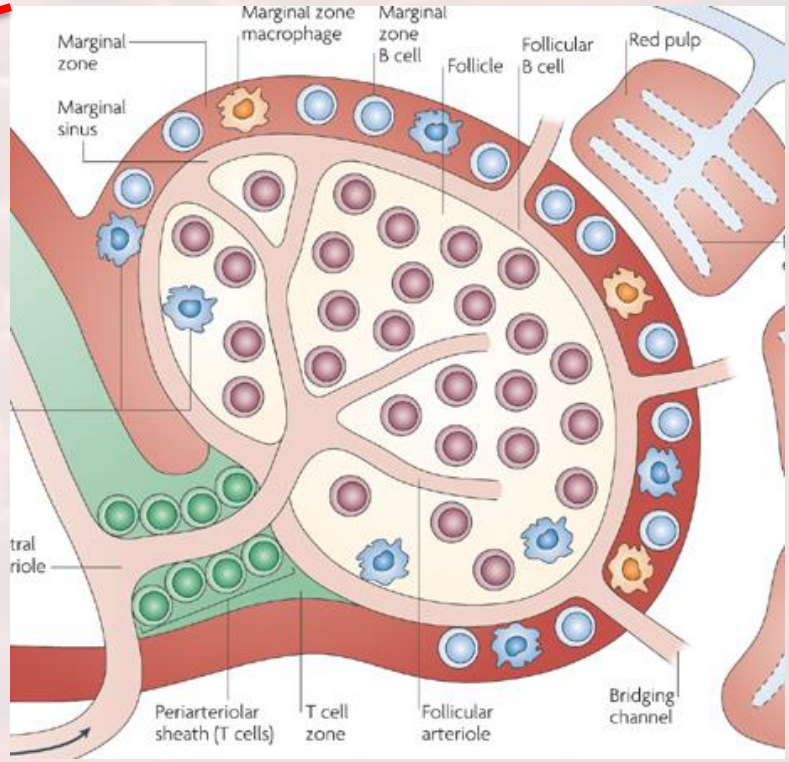
Murder on the Orient Express





Actual site of an immune response: very complex





Have animal models been useful?

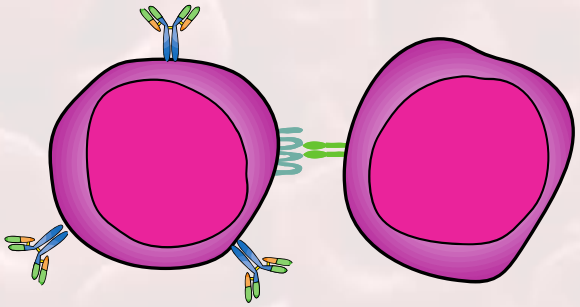
Discovery of B cells

(Max Cooper – chickens)

Discovery of antigenic variation between species

(Karl Landsteiner – mice, ducks, cows)

B cells



T cells

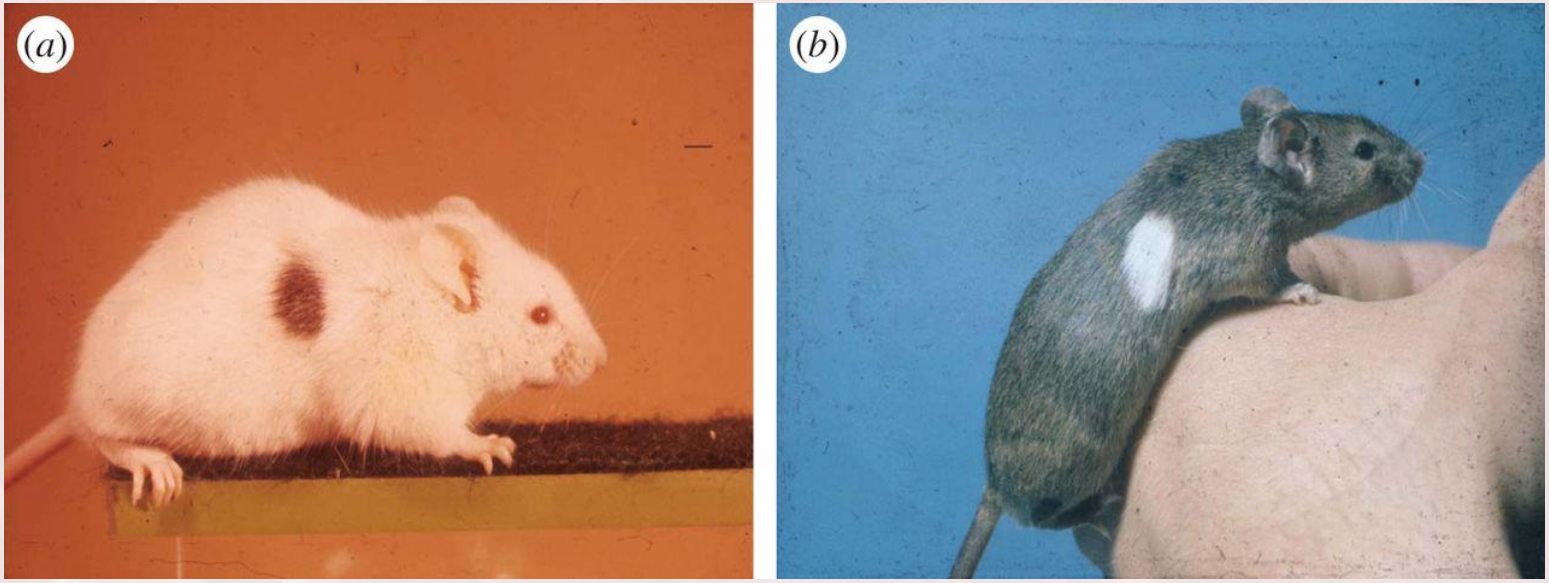
Discovery of the Major Histocompatibility complex

(George Snell – mice)

Discovery of T cell restriction

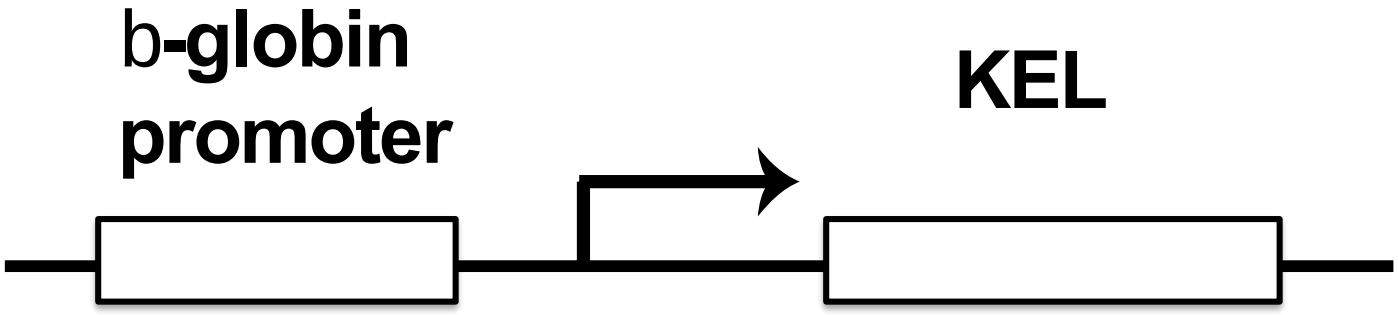
(Rolf Zinkernagel – mice)

Why have animal models not been used to study the immune consequences of transfusion?

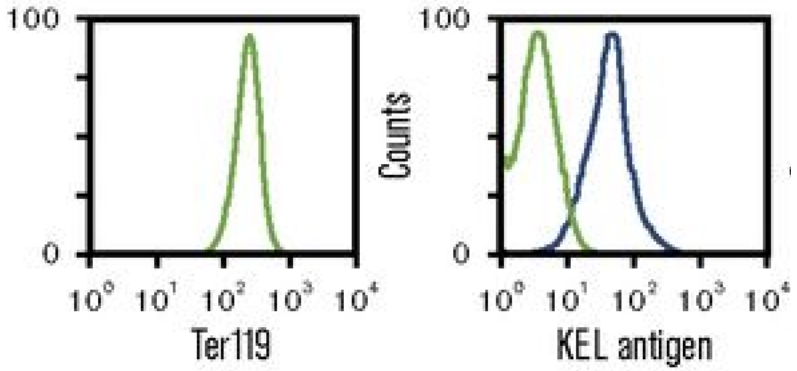


- Antigenic differences between mice can cause an immune response following transplantation.
- RBCs from different mice do not possess antigenic differences that routinely induce an immune response following transfusion.

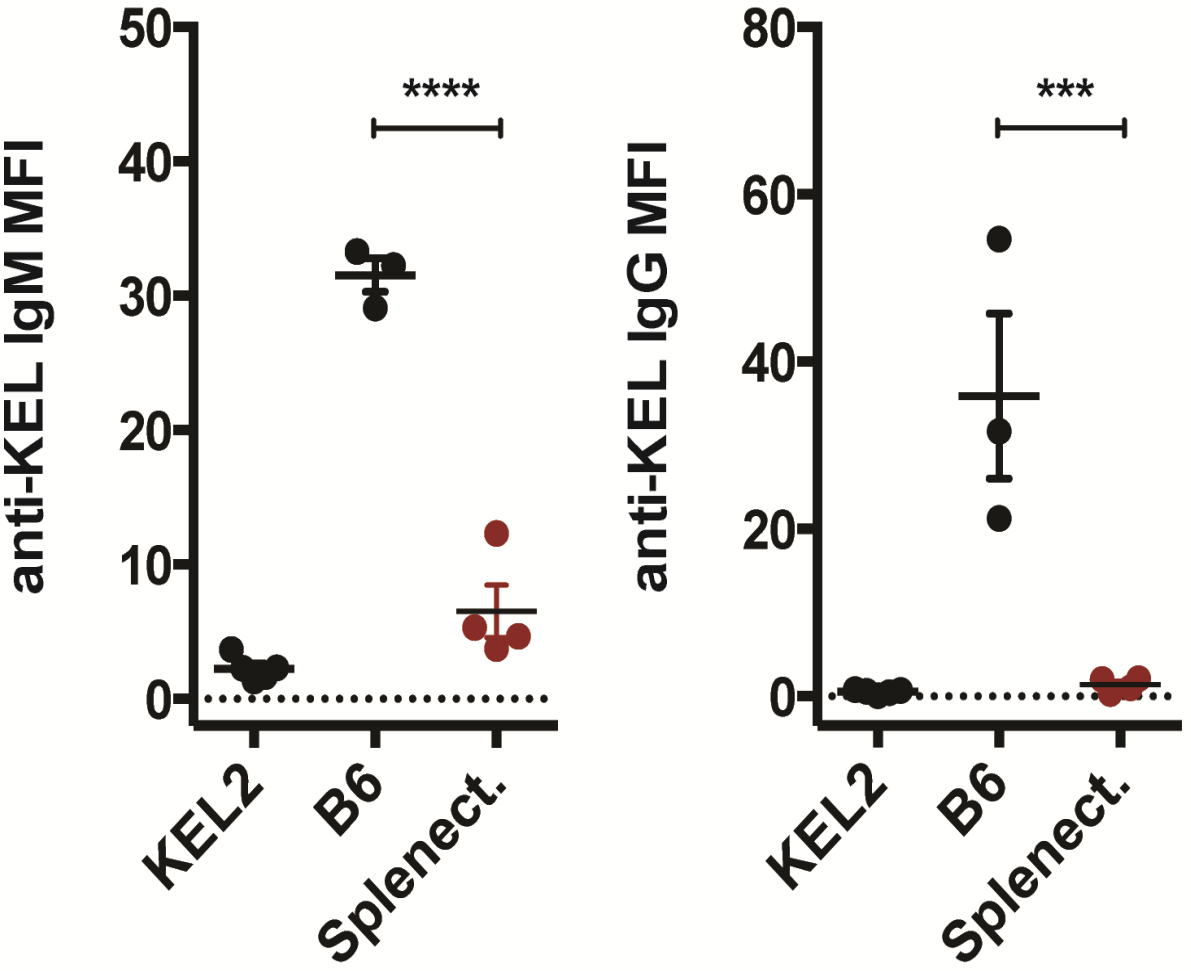
Using transgenics to generate models of RBC transfusion



KEL RBCs



Antibody response to transfused KEL RBCs is inhibited in splenectomized recipients



Spleen and transfusion

Is the spleen involved clinically?

- **What about sickle cell patients**
  - **Spleen function is not really known**
  - **Infection risk could be a result of many different factors**
  - **Hydroxyurea and transfusion cause regeneration of the spleen**

STUDIES ON THE ANTIBODY RESPONSE IN SPLENECTOMIZED PERSONS*

SAMUEL SASLAW, M.D., PH.D.,† BERTHA A. BOURONCLE, M.D.,‡ ROBERT L. WALL, M.D.,‡
AND CHARLES A. DOAN, M.D.§

COLUMBUS, OHIO

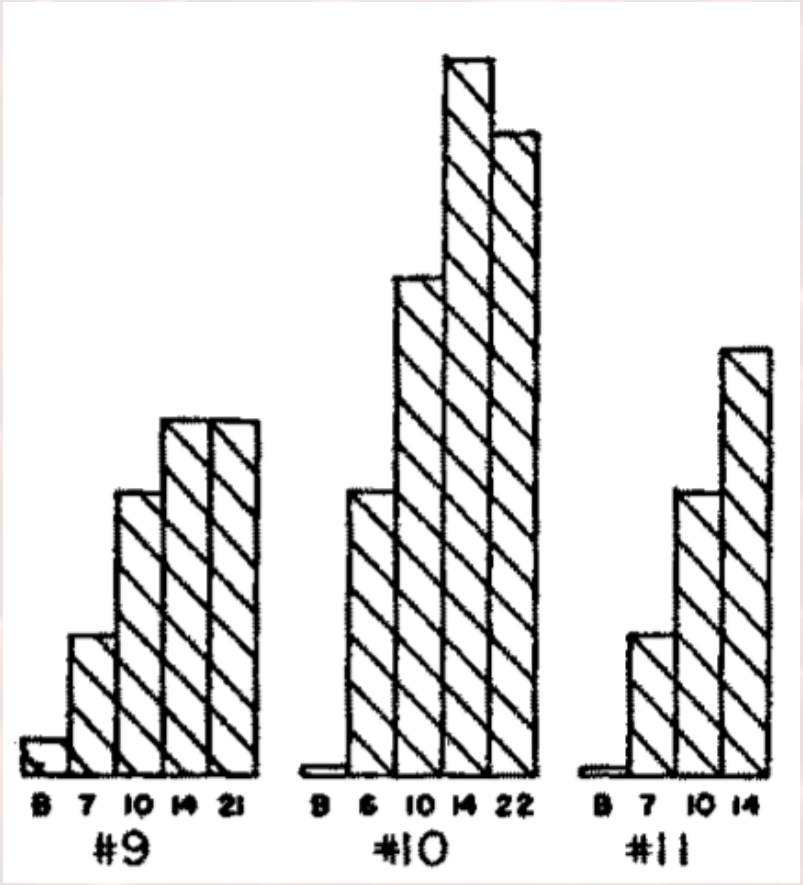
Saslaw et al, N Engl J Med 1959: 261;120-125.

Spleen and transfusion

Intact spleen

Splenectomized

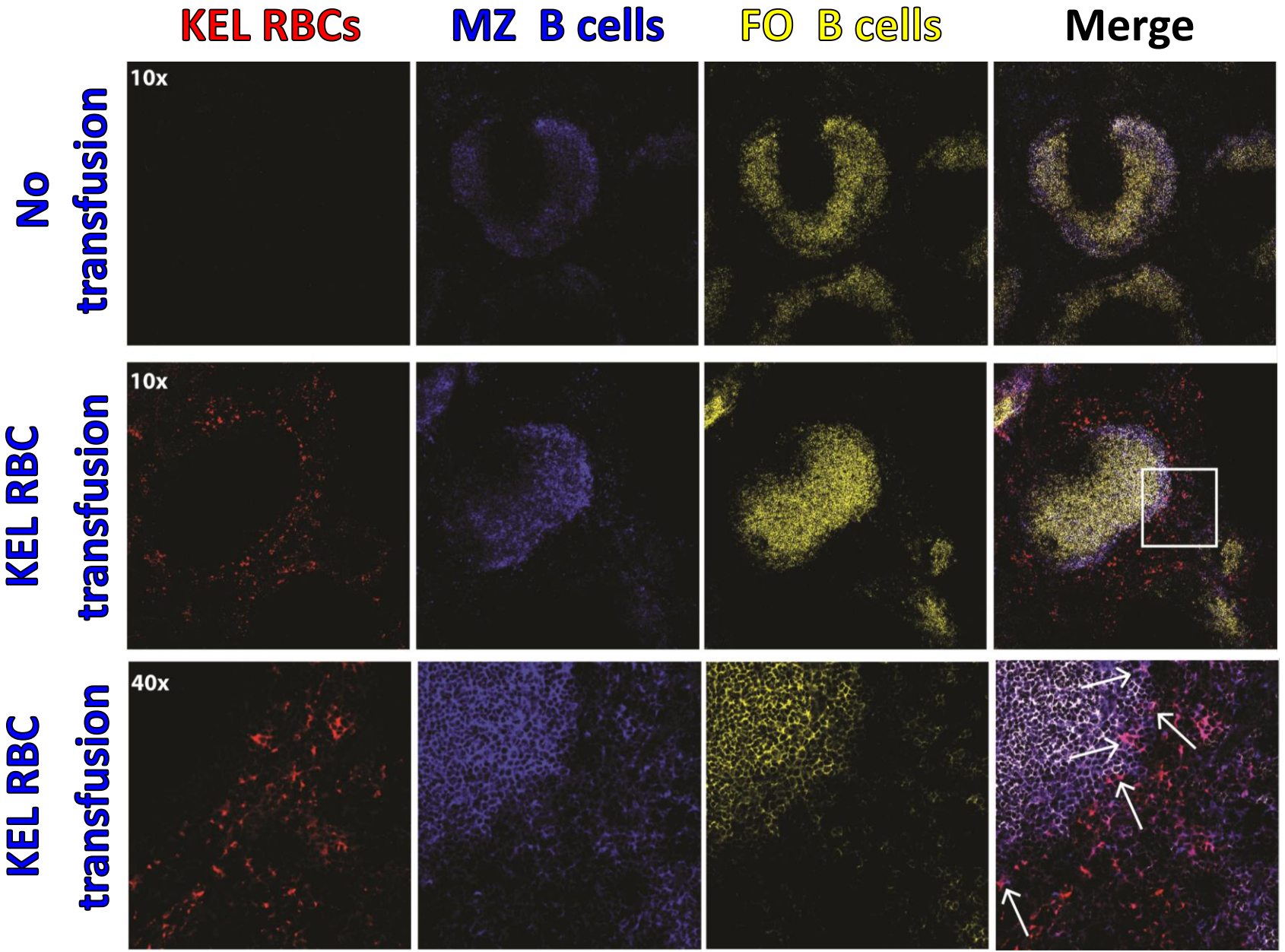
Anti-RBC antibody



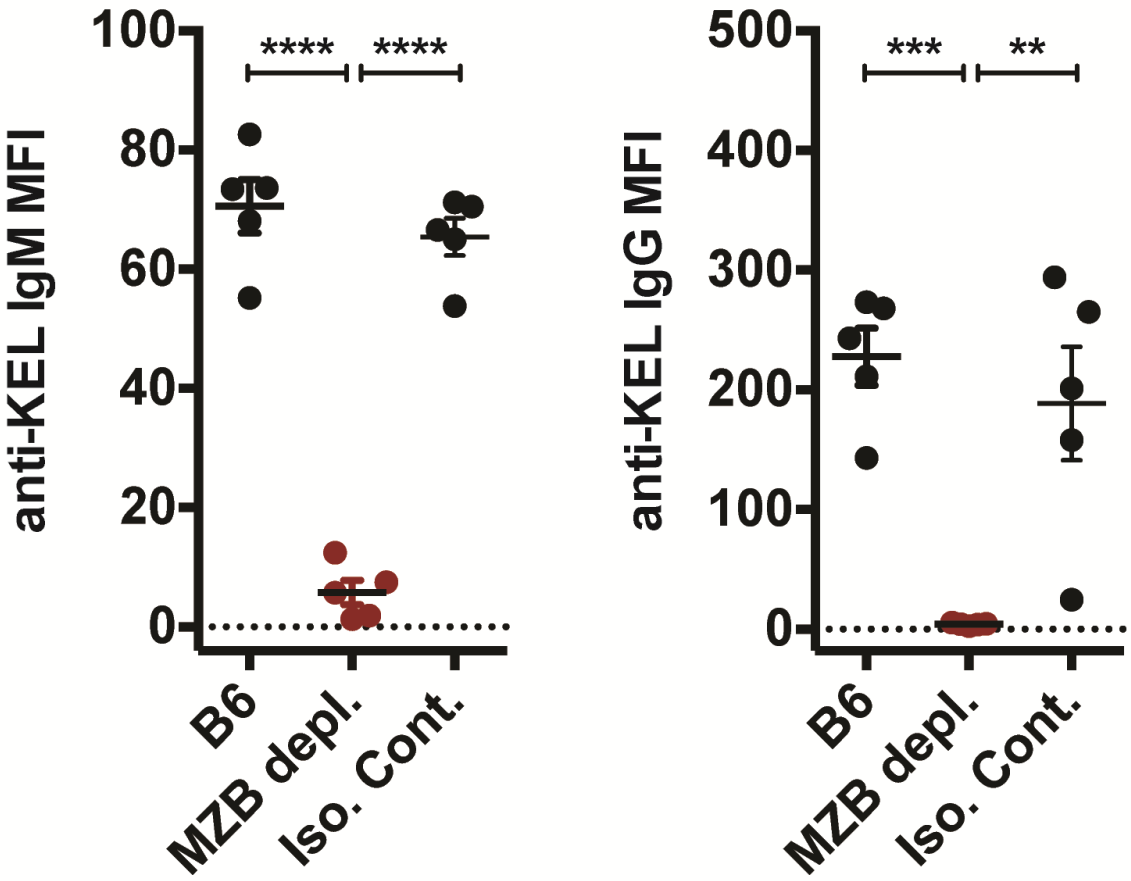
Evers et al. AJH 2017

Rowley et al, J Immuno 1950: 65;515-521.
Saslaw et al, N Engl J Med 1959: 261;120-125.

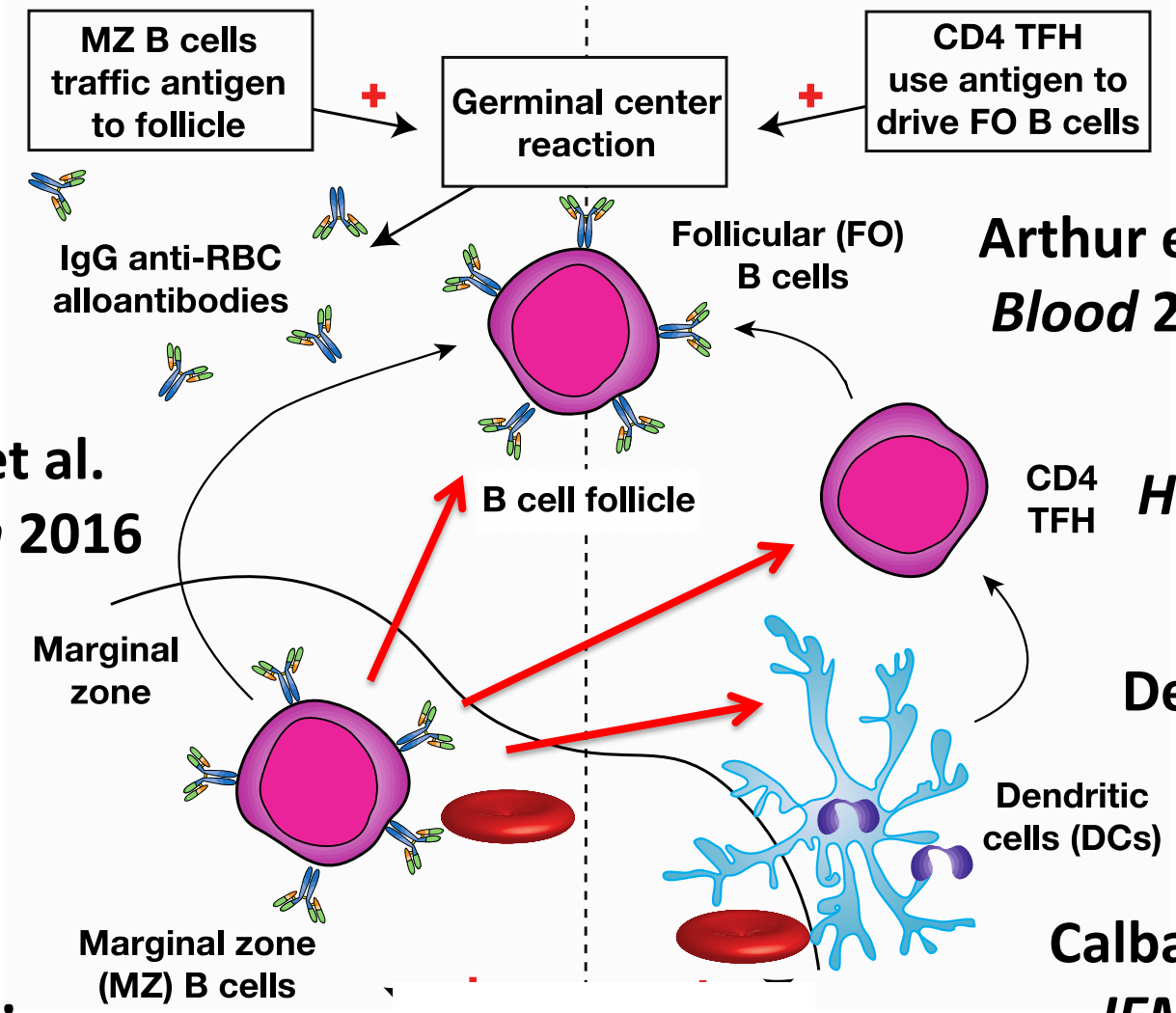
Transfused RBCs localize with marginal zone B cells



Depletion of MZ B cells prevents RBC alloimmunization



Model: Key players in RBC alloimmunization



Arthur et al. *Blood* 2016

Arneja et al. *Haematologica* 2016

Desmartes et al. *Blood* 2011

Calbaro et al. *JEM* 2016

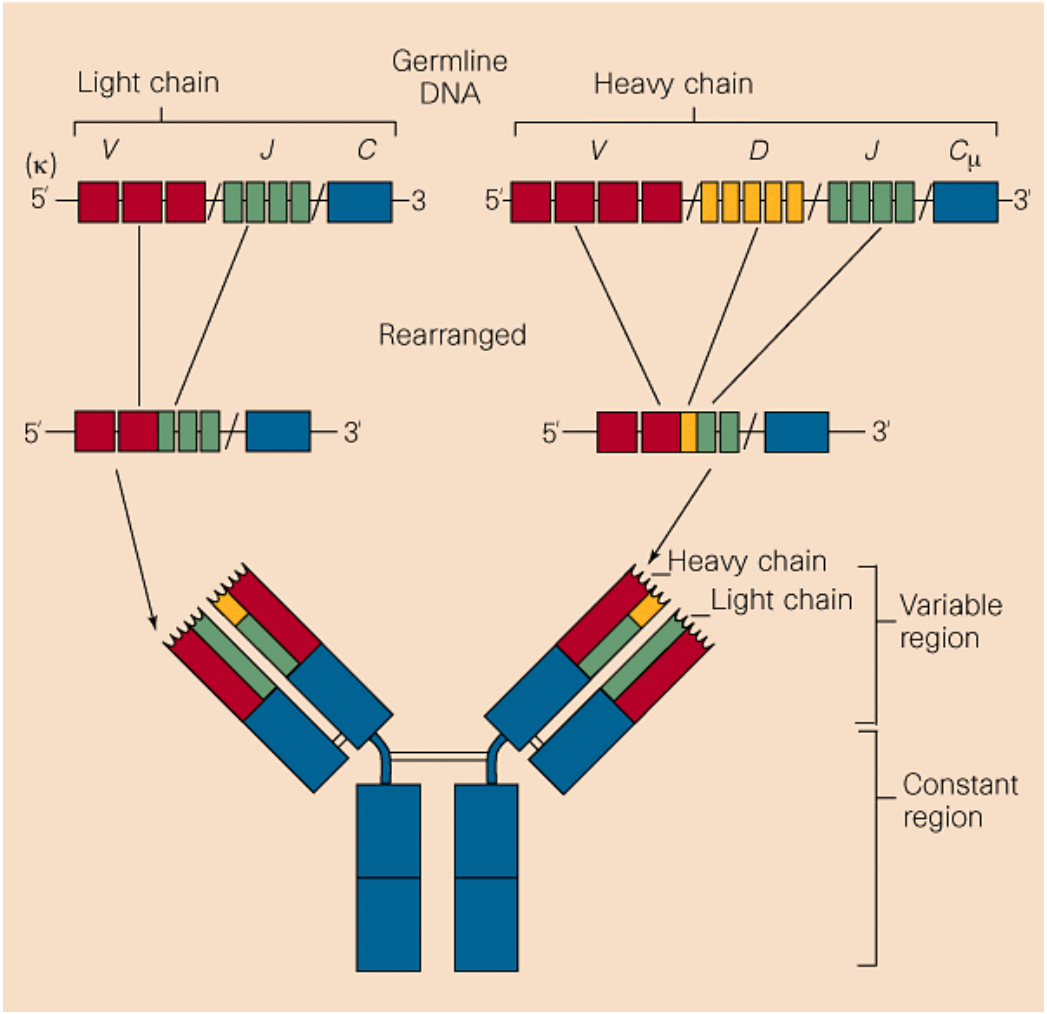
Zerra et al. *Blood* 2017

Liu et al. *Blood* 2017

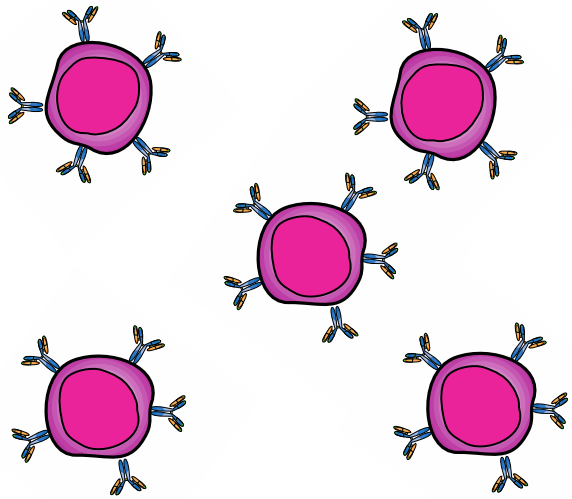
Gibb et al. *JI* 2017

Richards et al. *Transfusion* 2016

MZ B cells possess a more limited repertoire of antibody specificities

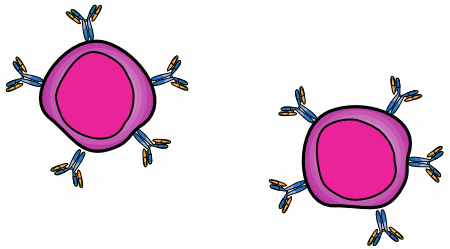


5 KEL-specific B cells



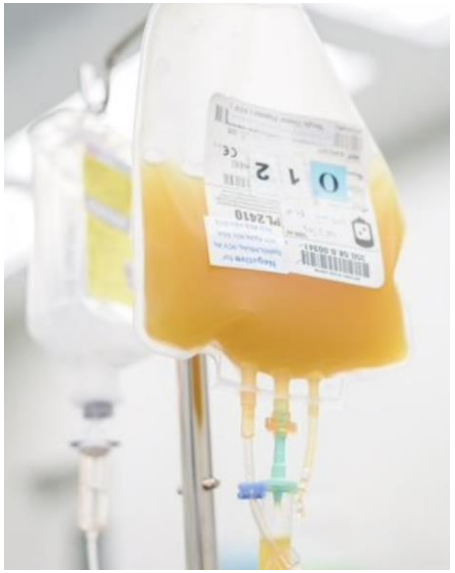
Responder

2 KEL-specific B cells



Non-responder

Platelet refractoriness



- 72 yo male with myelodysplastic syndrome
- Presented with mucosal bleeding
  - Platelet count < 4K/μL
- Received platelet transfusion
  - No change in platelet count (still < 4K/μL after transfusion)
- Primary team requested HLA-matched platelets
  - Anti-HLA alloantibodies detected
- Patient received HLA cross-matched platelets with an appropriate response

Platelet refractoriness

- **Inappropriately low platelet count increment following platelet transfusion (corrected count increment < 5000/m^2/μL)**
- **Immune-mediated:**
  - **Due to anti-HLA and/or anti-plate glycoprotein (HPA) alloantibodies**
    - **Treated with HLA or HPA matched or cross-matched platelets**
- **Non-immune:**
  - **Sepsis, Fever, Splenomegaly, Bleeding, DIC, etc.**
- **Some patients may appear to be refractory without a non-immune cause:**
  - **No anti-HLA and anti-platelet glycoprotein alloantibodies detected**
  - **Alloantibodies detected, but no response to HLA or HPA compatible platelets**

Platelet refractoriness

Response to Repeated Platelet Transfusion from the Same Donor

EMIL J. FREIREICH, M.D., ALLAN KLIMAN, M.D., LAWRENCE A. GAYDOS, M.D., NATHAN MANTEL, M.A., and EMIL FREI, III, M.D.

Bethesda, Maryland

Received February 27, 1963; accepted for publication May 13, 1963.

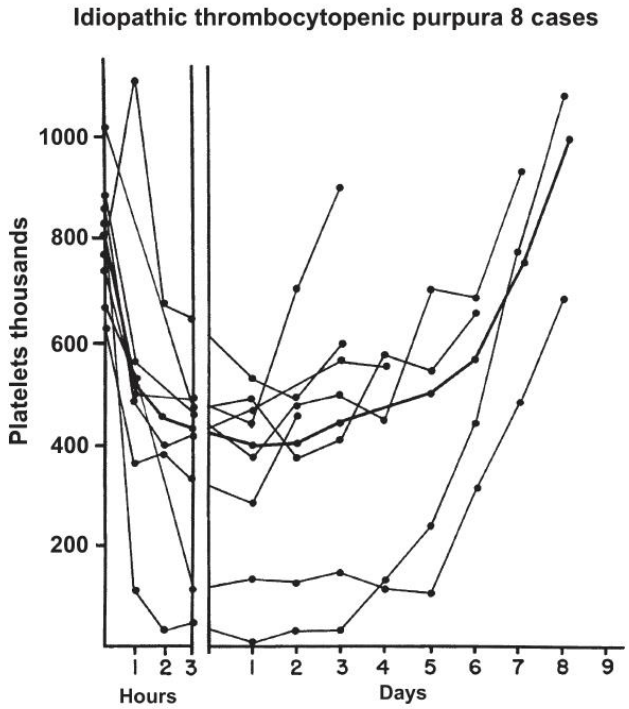
Complement-Fixing Platelet Iso-Antibodies in Serum of Transfused Persons. Correlation of Antibodies with Platelet Survival in Thrombocytopenic Patients

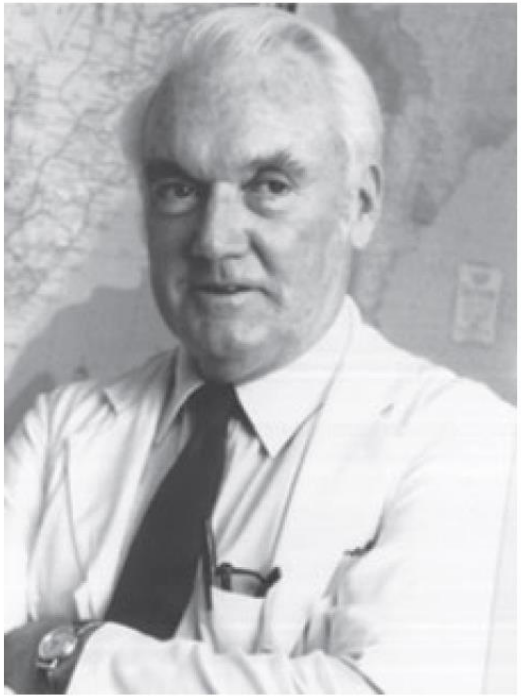
RICHARD H. ASTER,* ROBERT H. LEVIN,† HARVEY COOPER, EMIL J. FREIREICH

From the Division of Biologics Standards and Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Received for publication May 16, 1964; accepted June 25, 1964.

Immune Thrombocytopenia (ITP)





Passive administration of plasma from patients with ITP into healthy subjects resulted in transient thrombocytopenia (Harrington *et al.* J. Lab. Clin. Med. 1951)

Platelet refractoriness

Volume 280 APRIL 3, 1969 Number 14

SIGNIFICANCE OF THE POSITIVE CROSSMATCH TEST IN KIDNEY TRANSPLANTATION*

RAMON PATEL, M.R.C.P., AND PAUL I. TERASAKI, PH.D.

PLATELET TRANSFUSION THERAPY*

The Selection of Compatible Platelet Donors for Refractory Patients by Lymphocyte HL-A Typing

R. A. YANKEE, M.D., F. C. GRUMET, M.D., AND G. N. ROGENTINE, M.D.

Platelet refractoriness

CONCISE REPORT

High-Dose Intravenous Gammaglobulin in Alloimmunized Platelet Transfusion Recipients

By Charles A. Schiffer, Donna E. Hogge, Joseph Aisner, Janice P. Dutcher, Edward J. Lee, and Dottie Papenberg

Blood, Vol 64, No 4 (October), 1984: pp 937–940

1 out of 11 IVIg-treated patients exhibited an enhanced response to random donor platelet transfusion

VOLUME 337 DECEMBER 25, 1997 NUMBER 26

LEUKOCYTE REDUCTION AND ULTRAVIOLET B IRRADIATION OF PLATELETS TO PREVENT ALLOIMMUNIZATION AND REFRACTORINESS TO PLATELET TRANSFUSIONS

THE TRIAL TO REDUCE ALLOIMMUNIZATION TO PLATELETS STUDY GROUP*

Patients developed platelet refractoriness without detectable anti-platelet antibodies

Model of Platelet Refractoriness

Transfuse B6 recipients

Platelets: FVB

Check alloantibody levels

Transfuse:

B6 GFP platelets

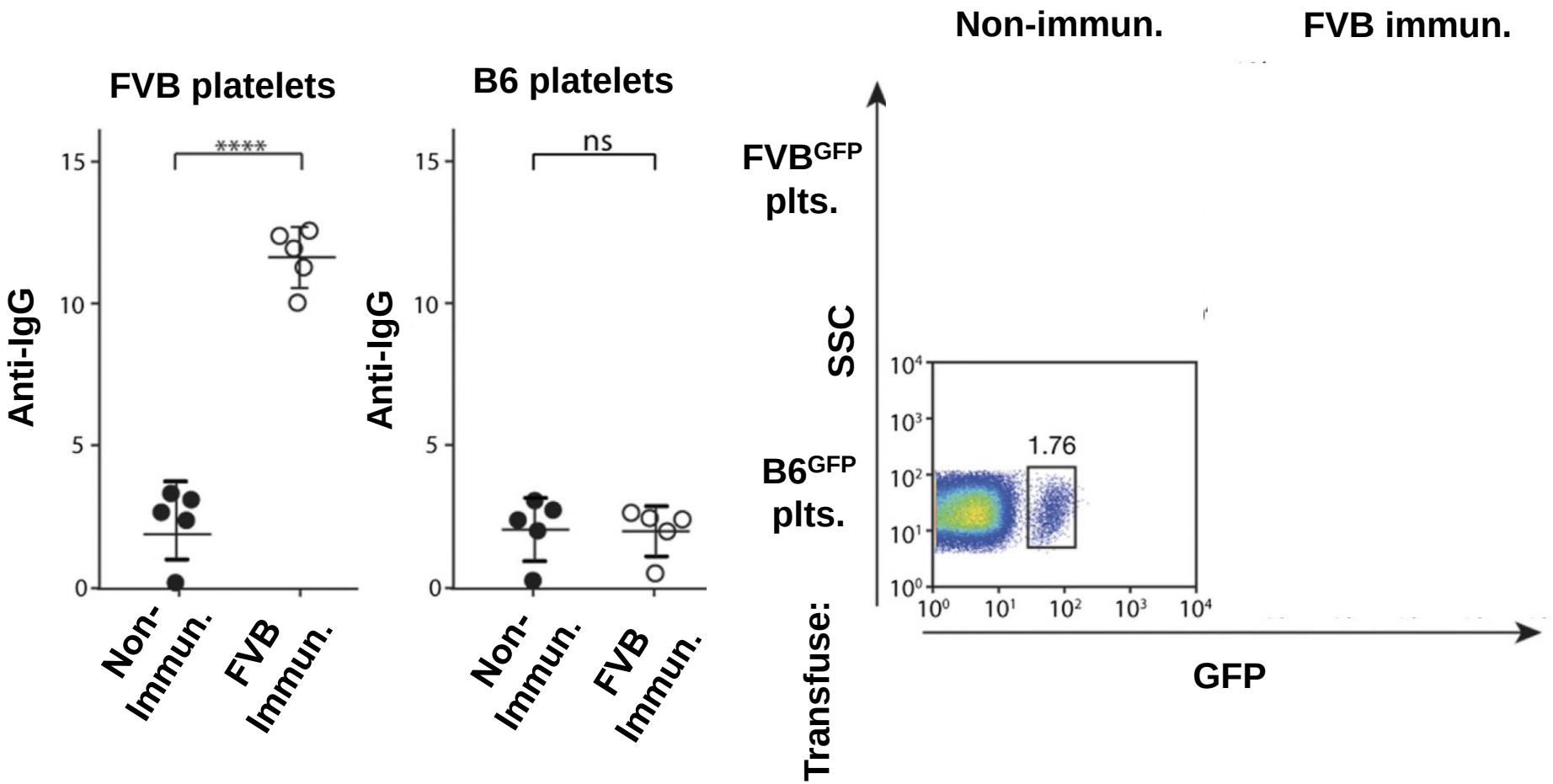
or

FVB GFP platelets

B6 class I: H-2Kb

FVB class I: H-2Kq

Modeling Platelet Refractoriness

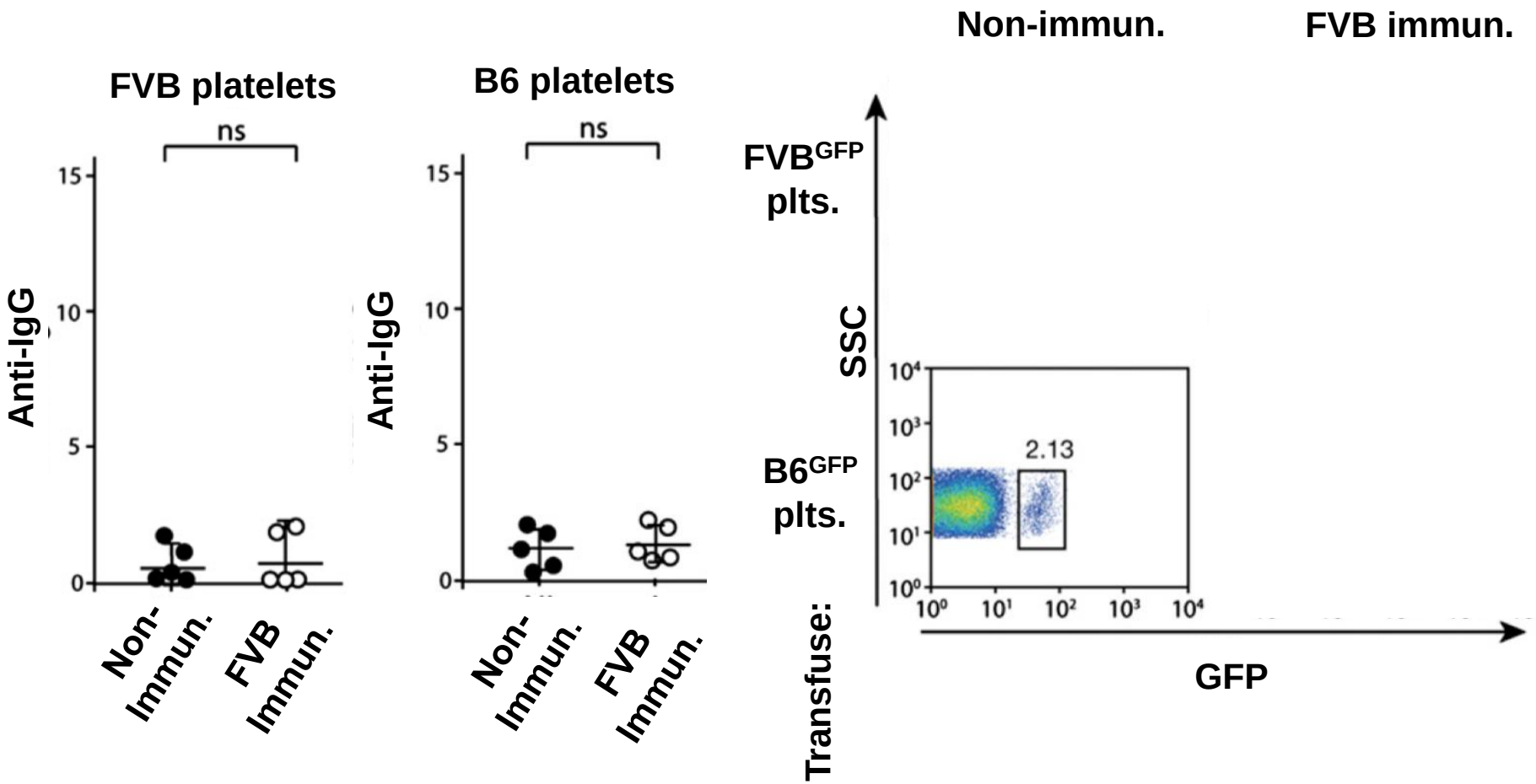


Immun. = Immunized

Recipients: B6 WT mice

plts. = platelets

Examining platelet refractoriness in B cell deficient recipients



Immun. = Immunized

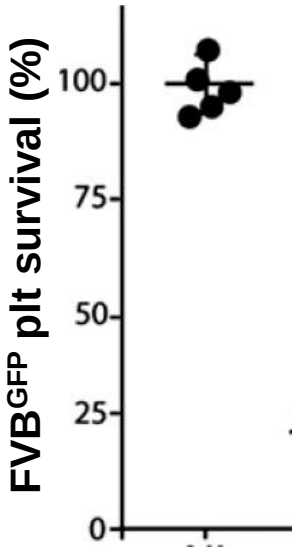
Recipients: B6 μMT B cell deficient mice

plts. = platelets

$CD8^+$ T cells can mediate platelet refractoriness

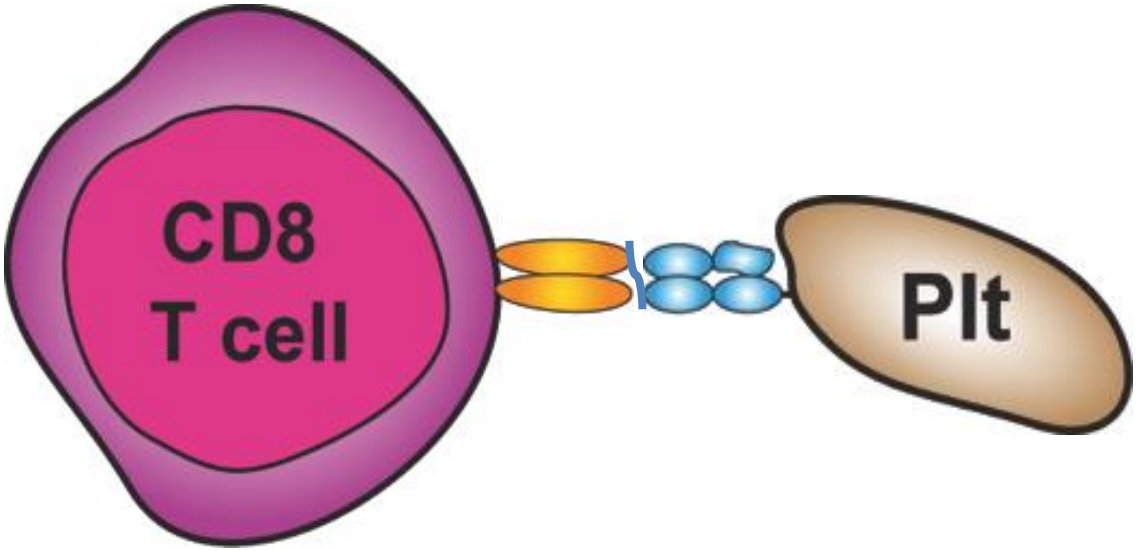
Recipients: B6 μMT B cell deficient mice

FVB platelets



FVB immunized	**-**
CD8 depleted	**-**
NK depleted	**-**

$CD8^+$ T cells can mediate platelet refractoriness

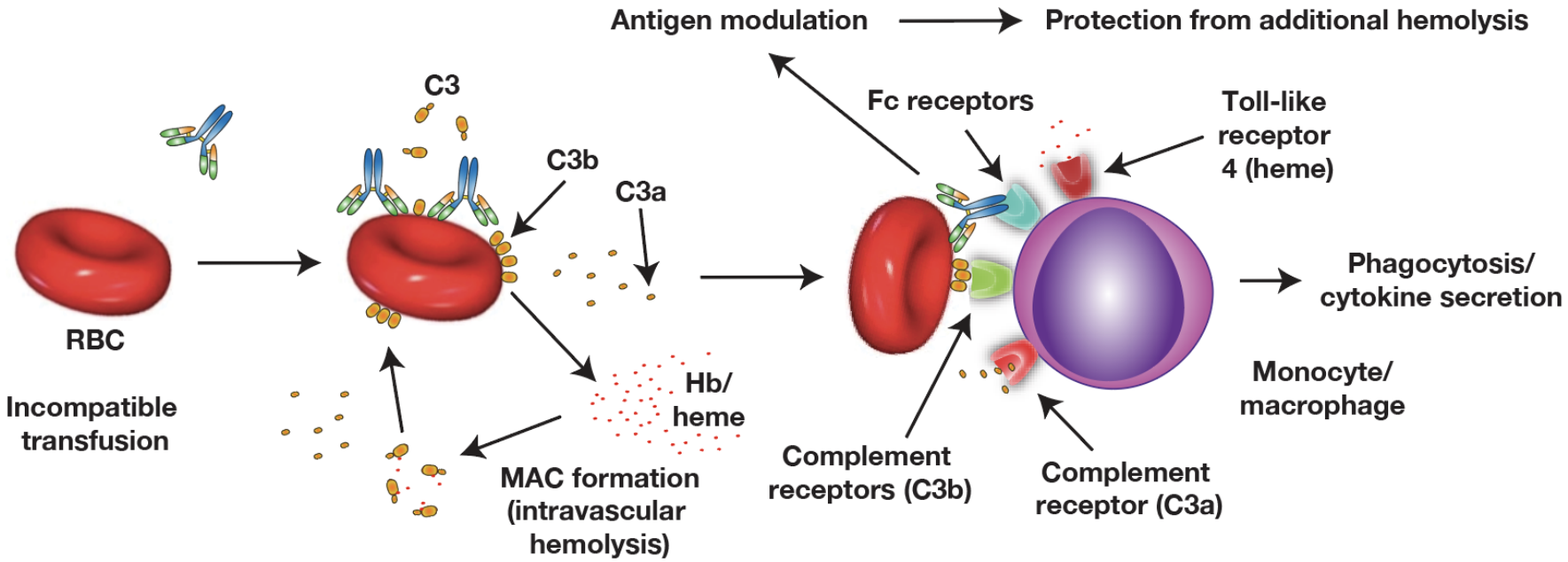


Incompatible RBC transfusion

Studies examining the consequence of incompatible transfusion

Sullivan et al.
Blood 2017

Patel et al.
Blood advances
2018

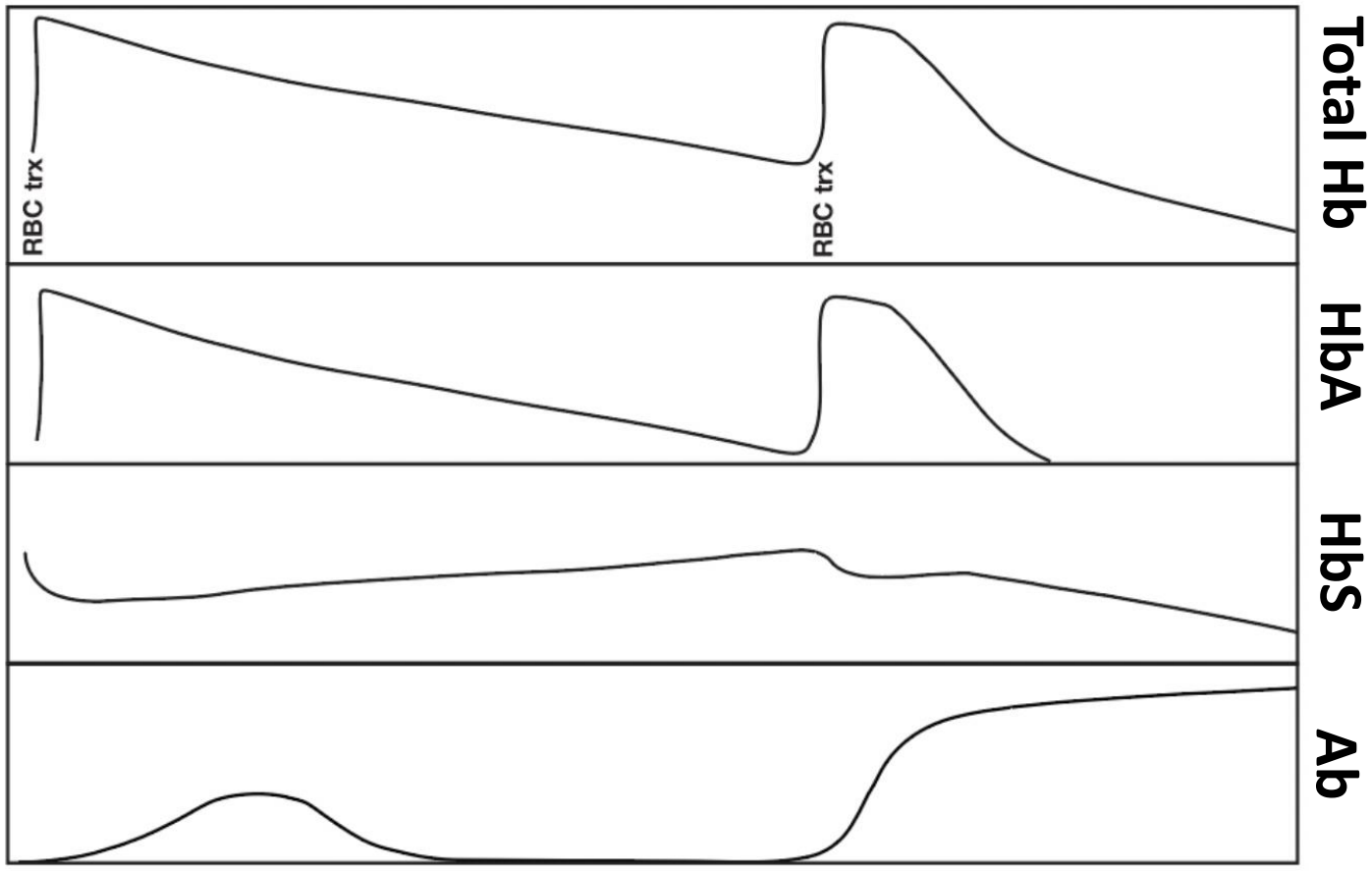


Mener et al.
Transfusion 2017

Arthur et al
Blood 2016

Maier et al.
Blood advances
2018

Delayed hemolytic transfusion reactions (DHTRs) and hyperhemolysis



Delayed hemolytic transfusion reactions (DHTRs)

BRITISH MEDICAL JOURNAL

LONDON SATURDAY MAY 18 1946

SOME RESULTS OF TRANSFUSION OF BLOOD TO RECIPIENTS WITH "COLD" AGGLUTININS

BY

K. E. BOORMAN B. E. DODD, B.Sc. J. F. LOUTIT, D.M.

AND

P. L. MOLLISON, M.D.

Report to Medical Research Council from the South-West London Blood Supply Depot

1180 THE NEW ENGLAND JOURNAL OF MEDICINE June 20, 1957

TRANSFUSION REACTIONS IN THE ABSENCE OF DEMONSTRABLE INCOMPATIBILITY*

HUGH FUDENBERG, M.D.,† AND FRED H. ALLEN, JR., M.D.‡

BOSTON

Hyperhemolysis

The Occasional Fallibility of *in Vitro* Compatibility Tests

HUGH CHAPLIN, JR., M.D., MONA CASSELL

From the Barnes Hospital Blood Bank and the Washington University School of Medicine St. Louis, Missouri

Received for publication February 26, 1962; accepted April 6, 1962.

A patient is described in whom rapid destruction of transfused red cells occurred repeatedly despite entirely compatible cross-match results by a wide variety of dependable laboratory procedures. The

Delayed type transfusion reactions in sickle cell disease

DHTRs in patients with sickle cell anemia: 1/1000 RBC transfusions

Delayed Hemolytic Transfusion Reaction Presenting as Sickle-Cell Crisis

W. JOHN DIAMOND, M.B., B.Ch.; FRANK L. BROWN, Jr., M.D.; PETER BITTERMAN, M.D.; HARVEY G. KLEIN, M.D.; RICHARD J. DAVEY, M.D.; and ROBERT M. WINSLOW, M.D.; Bethesda, Maryland

Annals of Internal Medicine. 1980; **93**:231-233.

Consequences of delayed hemolytic transfusion reactions with hyperhemolysis

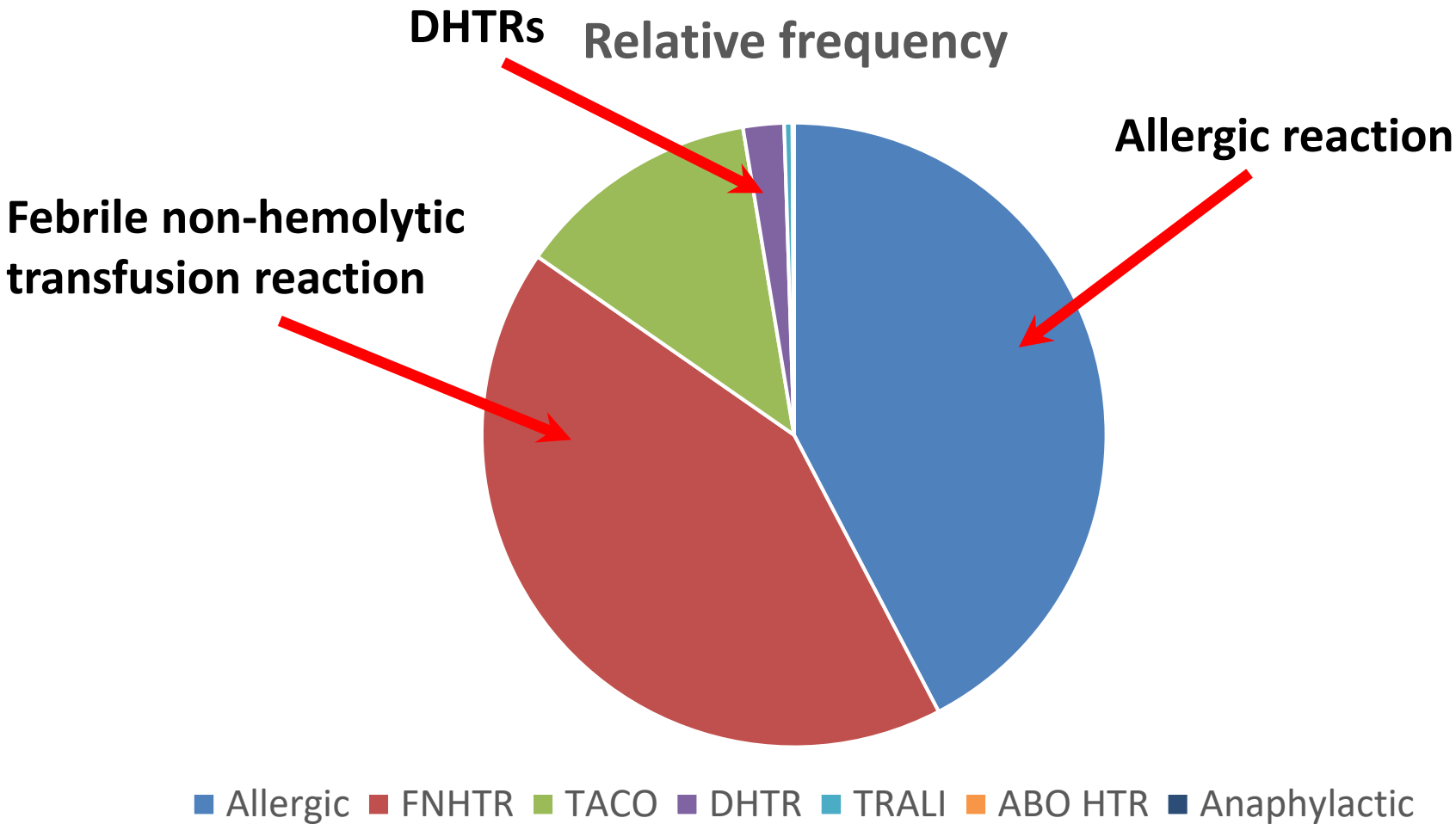
Incidence and predictive score for delayed hemolytic transfusion reaction in adult patients with sickle cell disease

David Narbey[1,2,3] | Anoosha Habibi[2,3,4] | Philippe Chadebech[1,2,3] |
Armand Mekontso-Dessap[5,6,7] | Mehdi Khellaf[7,8] | Jean-Daniel Lelièvre[7,9] |
Bertrand Godeau[5,10] | Marc Michel[7,10] | Frédéric Galactéros[2,3,4,7] |
Rachid Djoudi[1] | Pablo Bartolucci[2,3,4,7] | France Pirenne[1,2,3,7]

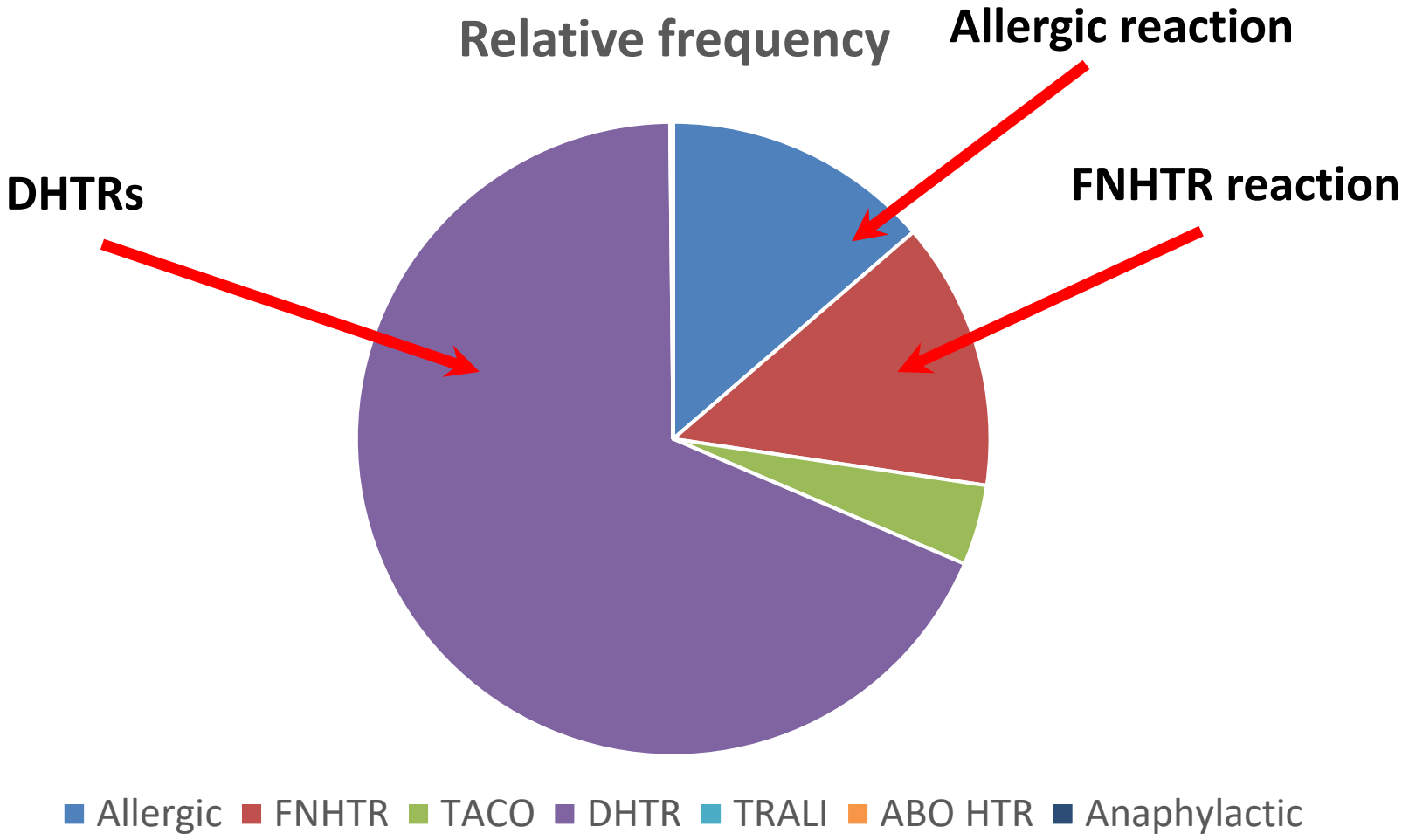
4.3% of all episodic transfusions resulted in a DHTR

11% of all patients who experienced a DHTR died

Current perceptions regarding the frequency of DHTRs



Changes in the perceptions of the frequency of DHTRs



Acute chest syndrome (ACS)

Research article

Extracellular hemin crisis triggers acute chest syndrome in sickle mice

Samit Ghosh, Olufolake Adetoro Adisa, Prasanthi Chappa, Fang Tan, Kesmic Ann Jackson, David Robert Archer, and Solomon Fiifi Ofori-Acquah

Aflac Cancer and Blood Disorders Center, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia, USA.

Satheesh Chonat

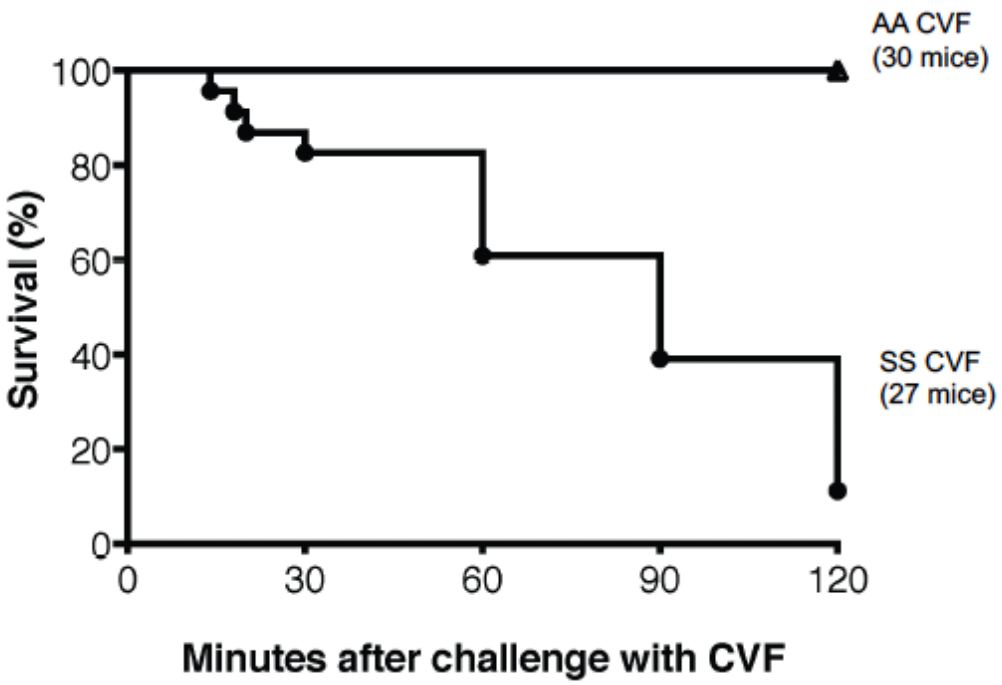
Hemin

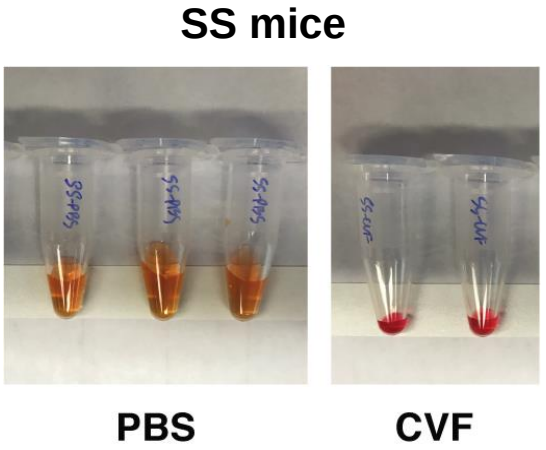
Examining the role of complement in acute chest syndrome

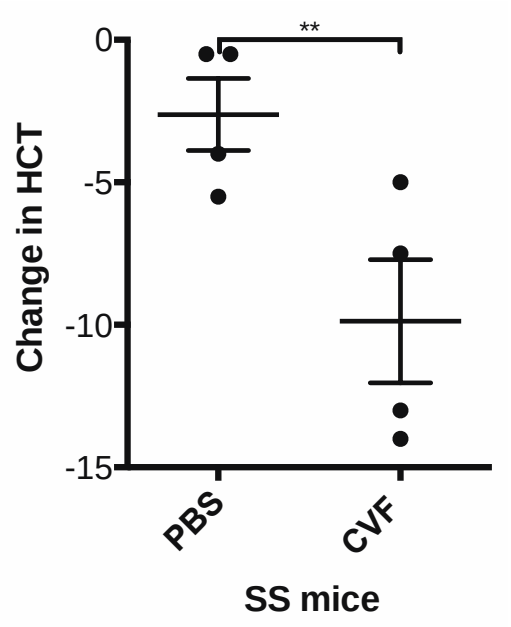
HbSS or HbAA mice treated with or without cobra venum factor (CVF)

Evaluate for acute chest syndrome

Sickle cell mouse response to cobra venom factor (CVF)

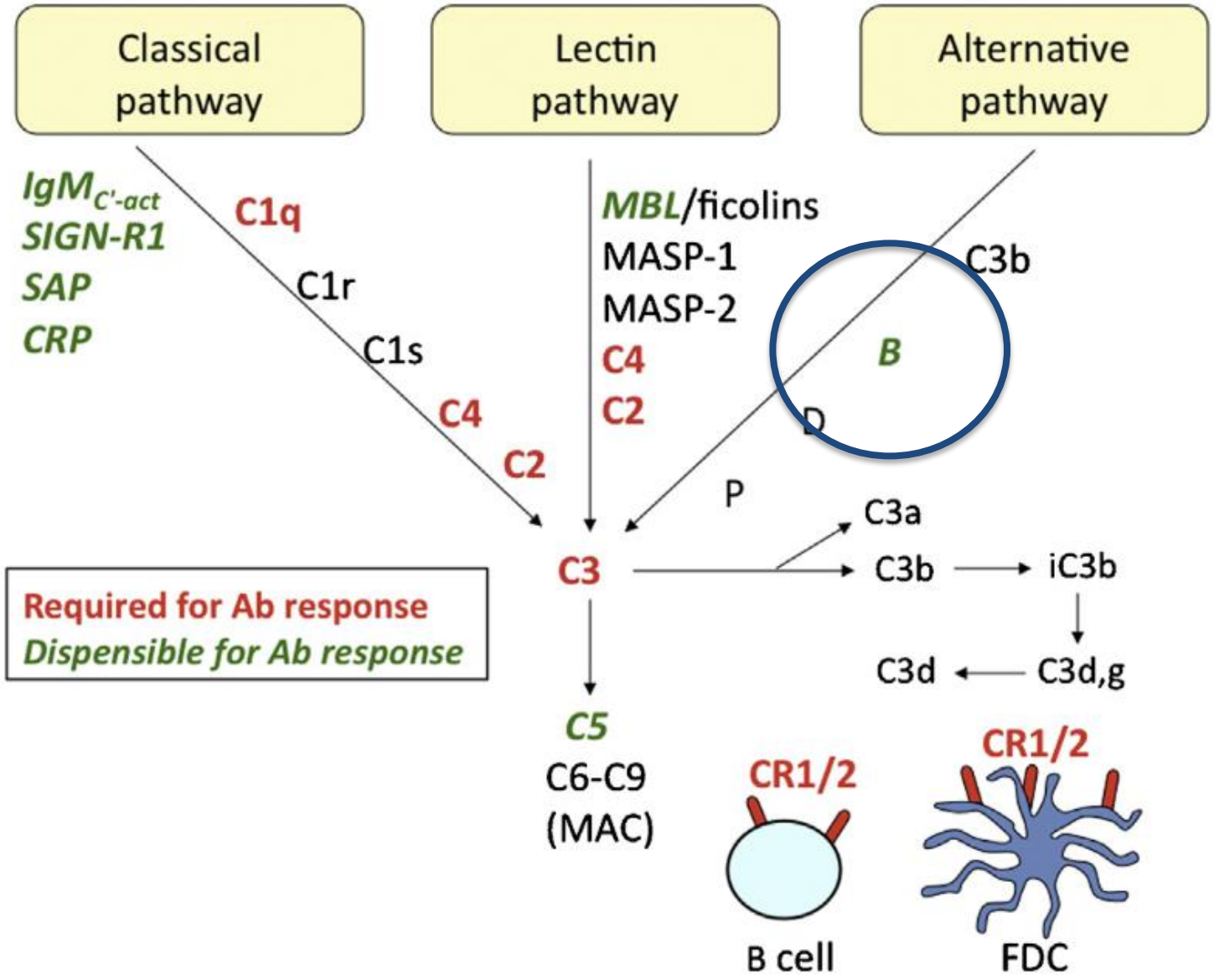






Evaluation of alternative complement activation in patients with ACS

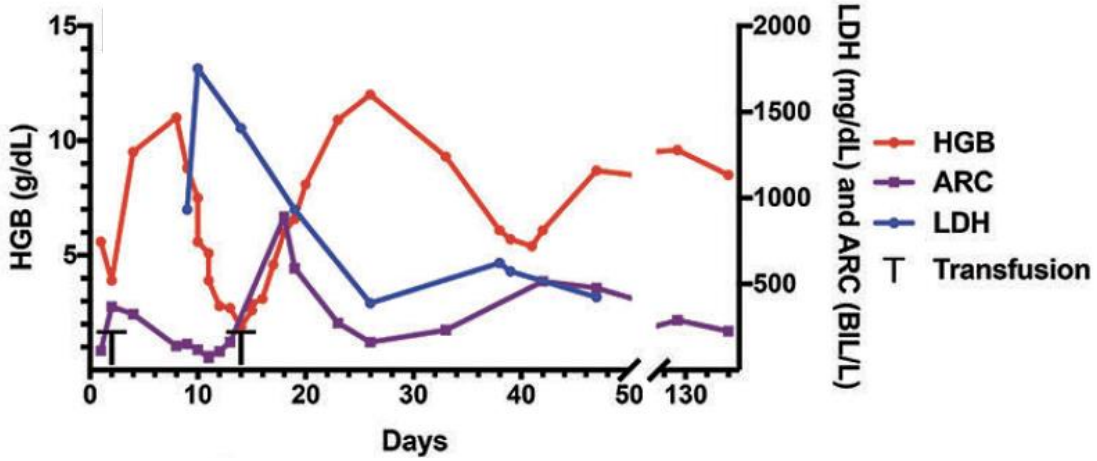
Alternative complement activation during ACS



Treatment of hyperhemolysis with eculizumab (C5 inhibitor)?

14 yo female with sickle cell disease

Bb (μg/ml): 0.95 6.06 0.96



Steroids
Methylprednisone 2 mg/kg/day

Erythropoietin
300 IU/kg x3/week to daily

Rituximab
375 mg/m^2

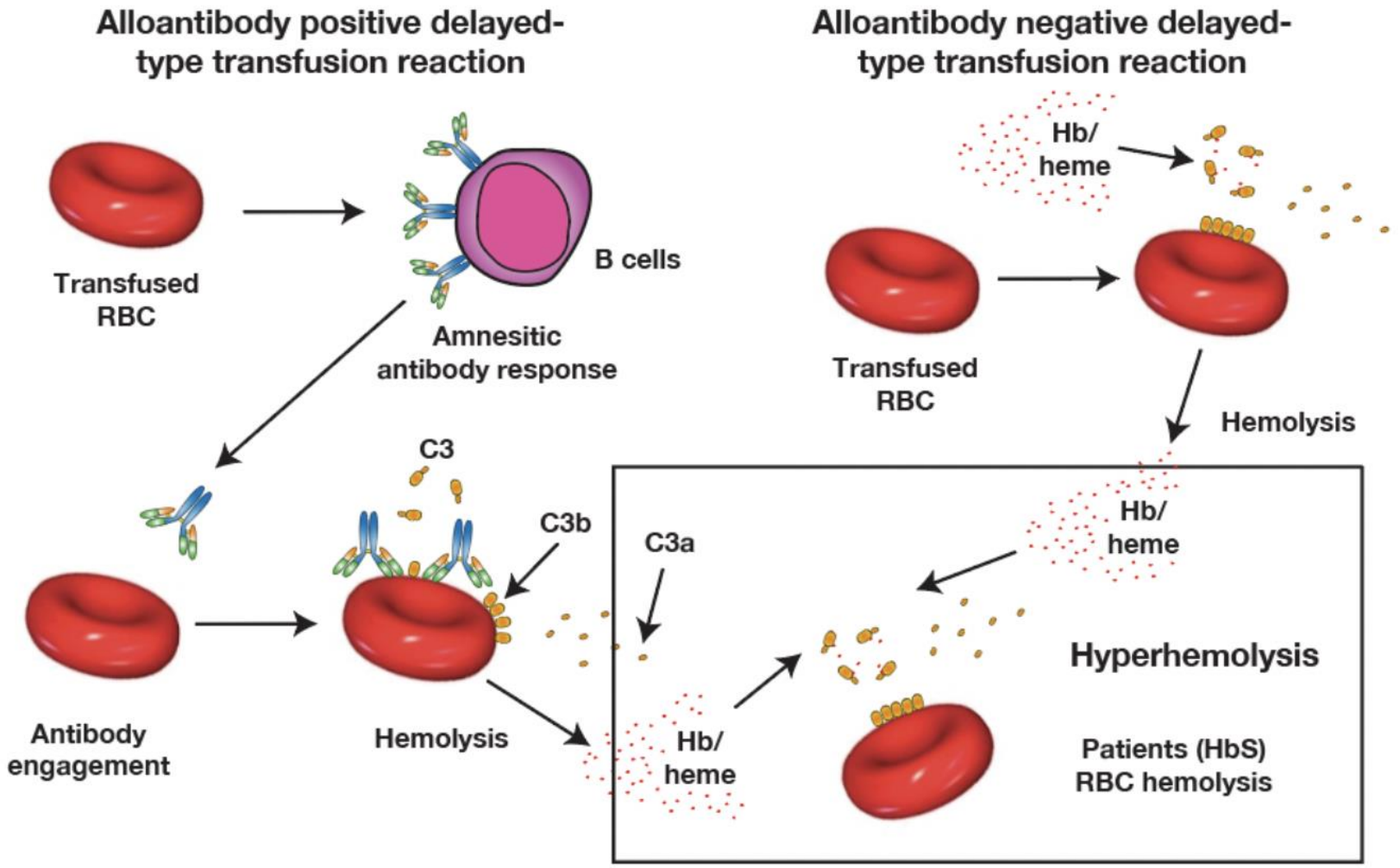
Eculizumab
600 mg

HGB: hemoglobin

ARC: absolute reticulocyte count

LDH: lactate dehydrogenase

Delayed hemolytic transfusion reactions (DHTRs) and hyperhemolysis



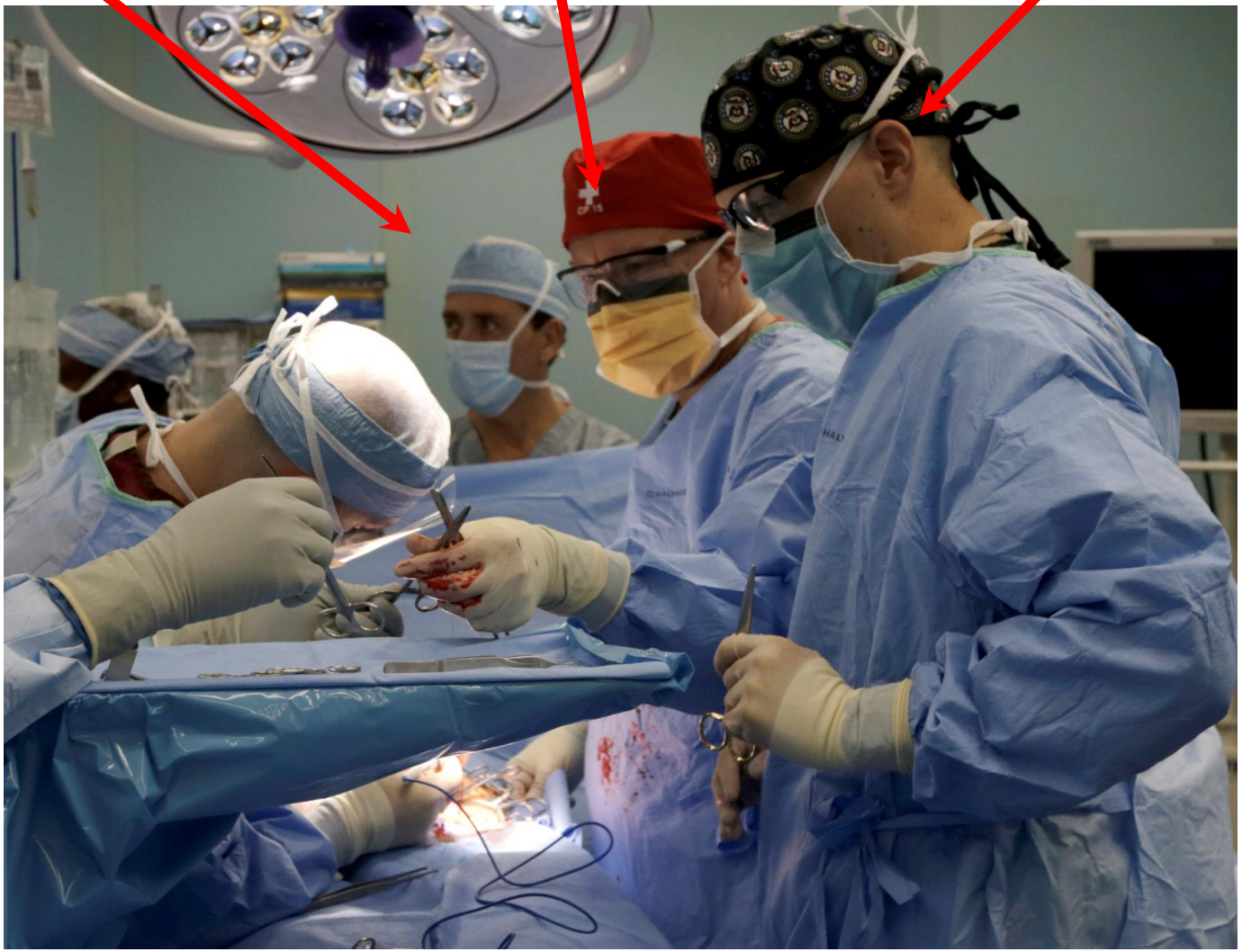
Working together to help patients (now and efforts to improve care in the future)

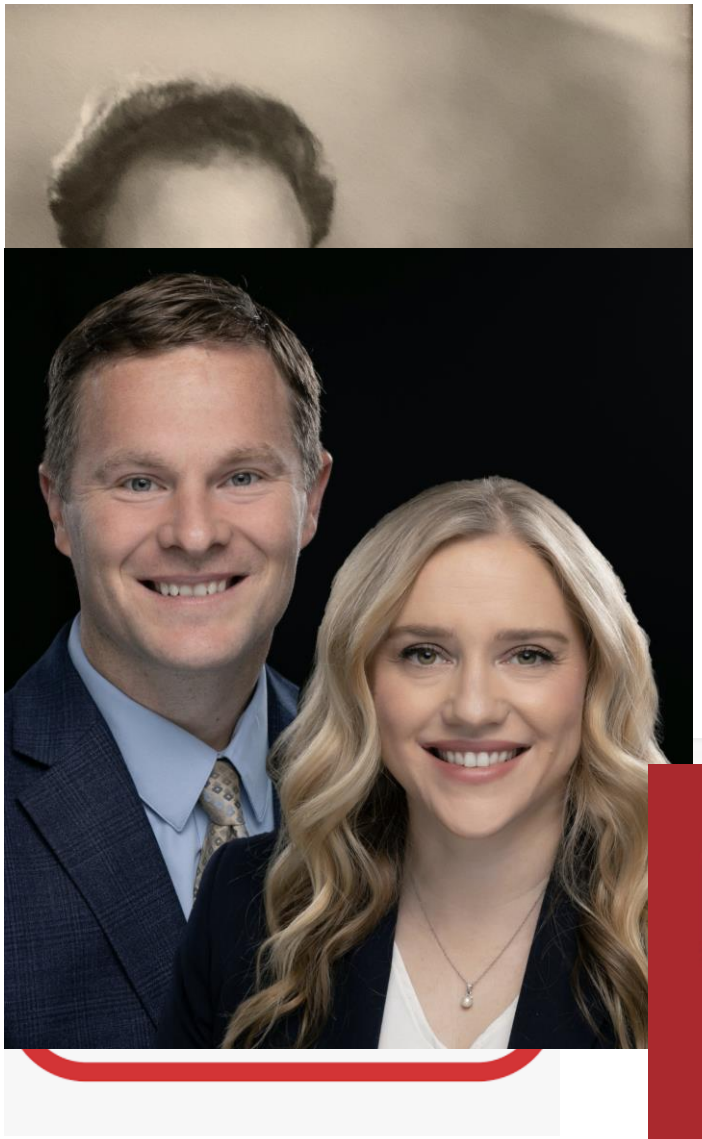
Working together to help patients (now and efforts to improve care in the future)

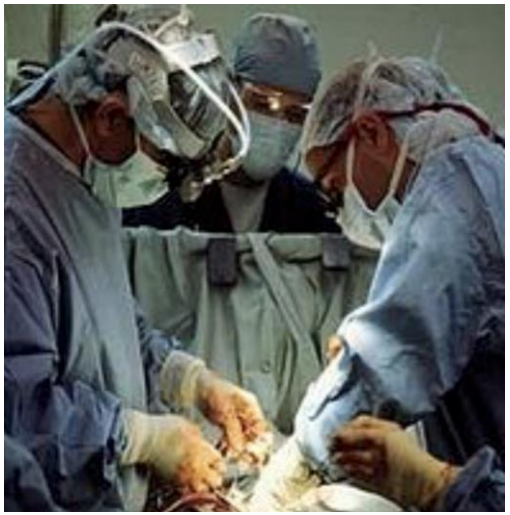
Basic scientist

Translational researcher

Clinician





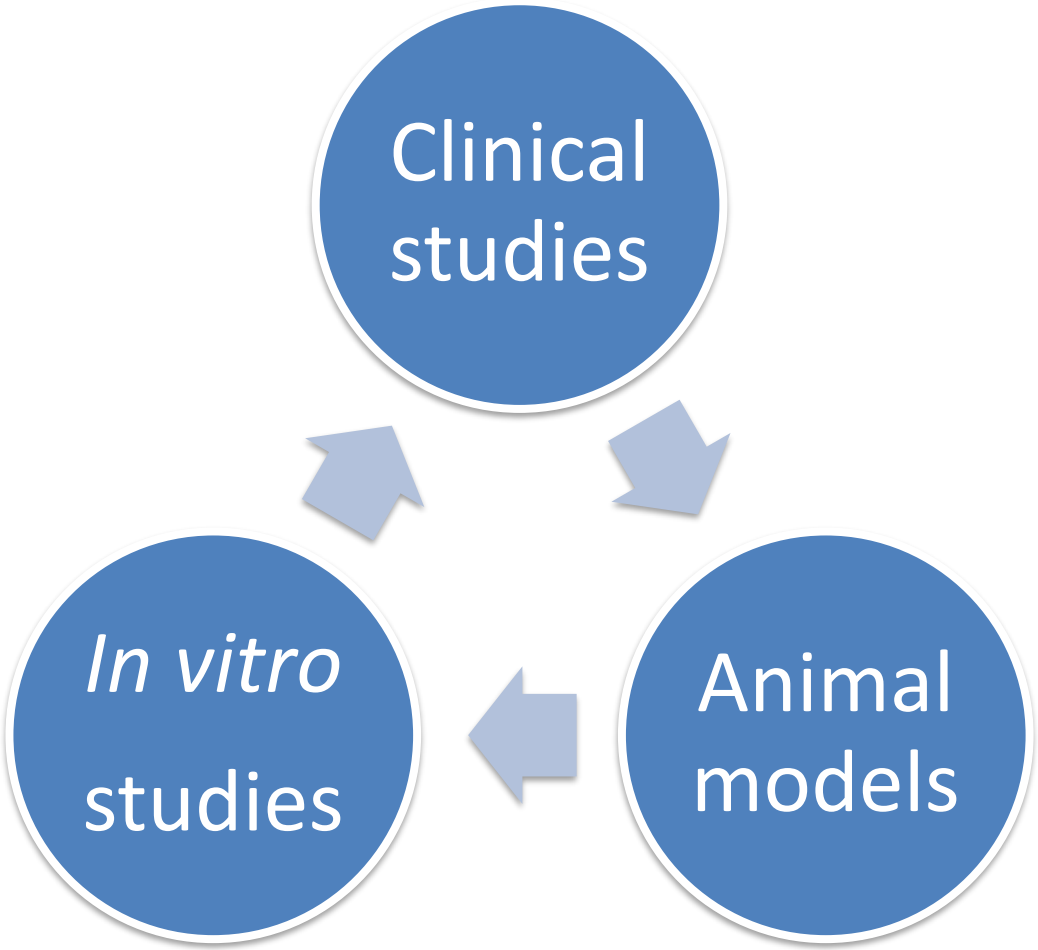


Chemical engineer

Anesthesiologist

Surgery (General, Trauma and CT)

Biomedical research: Iterative process with many different "specialties" that work together to enhance future care



Translating basic science research

Richard Cummings

P-selectin discovered (1984)

Ligand discovered (1992)

P selectin KO (2002)

Drug developed (2010)

Phase 2 clinical studies (2013)

32 years!

Translating basic science research

The NEW ENGLAND JOURNAL of MEDICINE

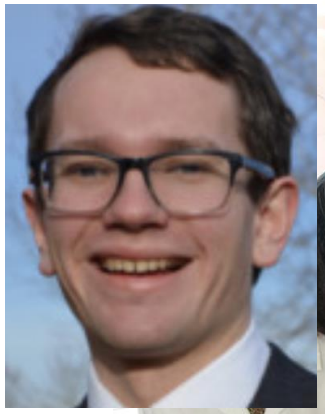
ORIGINAL ARTICLE

Crizanlizumab for the Prevention of Pain Crises in Sickle Cell Disease

K.I. Ataga, A. Kutlar, J. Kanter, D. Liles, R. Cancado, J. Friedrisch, T.H. Guthrie, J. Knight-Madden, O.A. Alvarez, V.R. Gordeuk, S. Gualandro, M.P. Colella, W.R. Smith, S.A. Rollins, J.W. Stocker, and R.P. Rother

New Novartis medicine Adakveo® (crizanlizumab) approved by FDA to reduce frequency of pain crises in individuals living with sickle cell disease

Ataga et al. *NEJM* 2017



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