It’s not just allo-antibodies that a red cell transfusion can stimulate

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Transfusion practice

• Minimise risk of transmitting disease
  – Donor selection and testing
  – Leucodepletion
  – Bacterial testing of platelet products
  – Viral neutralisation

• Minimise risk of haemolytic transfusion reaction
  – Match for ABO, RhD and other red cell antigens in selected cases
  – Screen for and identify atypical antibodies if present
  – Crossmatch antigen negative donor units
Residual risk

• ARCBS indicates residual risk of contracting an infectious agent for which they test is less than one in a million.
• No residual risk data published for likelihood of ATR or chronic TR
• haemovigilance data does indicate the frequency of a number of events occurring following transfusion

Antibody ID and crossmatch can’t guarantee

• all ABO mismatches are detected
• RhD typing mistakes will be detected
• transfused rbcs will have normal survival
• that recipient will not have a reaction
• that recipient will not produce antibodies to transfused blood
Primary and secondary immune responses

**Primary challenge**
- Long latent period
- No memory cells
- Large immunogen dose
- Predominantly IgM some IgG
- Low avidity antibody
- Low titre antibody

**Secondary challenge**
- Short latent period
- Many memory cells
- Small immunogen dose
- Predominantly IgG some IgM
- High avidity antibody
- High titre antibody

**T-cell independent antigens**

- Simple biochemistry
- Repeating identical epitopes
- Most often soluble
- B1-cell mitogens
- Little immunological memory
- Stimulate IgM ‘cold reacting’ antibodies

Polysaccharides, lipopolysaccharides
Carbohydrate based blood group antigens
- AB, H, I, Le, P/Pₖ, MN
T-cell independent antigens: Immune response

- **B1-cell**
- **Plasma cell**

- Differentiation
- Division

- Secreted IgM antibody

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T-cell dependent antigens

- Complex biochemistry
- Mostly particulate eg. on cells
- Many different epitopes
- Require ‘processing’
- Long lasting immunological memory
- Stimulate IgM and IgG warm reacting antibodies
  - Proteins, glycoproteins
  - RH, K, Fy, Jk, etc.
  - Carbohydrate dependent antigens if RBC associated
T-cell dependent antigens: Immune response

Blood group antigen immunogenicity

<table>
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<th>Antigen</th>
<th>Immunogenicity %</th>
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<td>D</td>
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<td>S</td>
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Antigen processing and presentation

• T-cell dependent antigens

• Ability to present exogenous epitope maybe controlled by class II genotype eg. HPA-1a, DRB3*0101 neg individuals cant process HPA-1a ag

Specificity of immune response

  – Recognised that autologous rbcs could be lysed by non-specific mechanisms
  – Accidental fit of abnormal or cross-reacting protein
  – Innocent bystander reaction is transient and not self-perpetuating

• Thompson RA, Rowe DS. Reactive lysis – a distinct form of red cell lysis. Immunology 1968;14:745-762
  – Innocent bystander lysis due to C5a67 attaching to autologous rbcs.
Specificity of immune response

• Schonewille and Brand. Does an alloimmune response to strong immunogenic red blood cell antigens enhance a response to weaker antigens? Transfusion 2008;48:958-963
  – High responders don’t make multiple antibodies

  – 68% of Rh negative volunteers developed anti-D
  – Pool of anti-D had antibody that reacted with Rh negative cells
  – Two volunteers developed positive DATs

Specificity of immune response

• McGrath et al Transient platelet and HLA antibody formation in multitransfused patients with malignancy BJH 1988;68:345-350
  – 28/59 patients developed transient antibodies to platelet specific antigens, often associated with infection and 50% demonstrated autoantibody activity

• Young et al. Autoantibody formation after alloimmunisation: are blood transfusions a risk factor for autoimmune haemolytic anemia. Transfusion 2004;44:67-72
  – 4.6% of 2618 transfused patients with positive IAT or DAT had rbc autoantibodies, 41/121 had both allo and auto antibodies remainder only autoantibody. 12/41 developed autoantibody in association with alloimmunisation following transfusion
Specificity of immune response

  - Patient developed anti-c following transfusion of two units of ABO and RhD matched blood. Following transfusion of two units of ABO matched R,R, K:-1 units patients Hb dropped from 92g/L to 53 g/L and had positive DAT. Eluate and IAT reacted with all panel cells. Had also developed an allo anti-Jk in addition to the anti-c. Conclusion bystander immune hemolysis.

Antibody production in transfused patients

2490 transfused patients
↓

2082 had post-transfusion follow-up
↓

78 patients with evidence of alloimmunization post-transfusion (+DAT and/or +IAT)
↓

4 patients
  passive antibody
  Anti-A (2)
  Anti-Kp' (1)
  Anti-K (1)
↓

15 patients
+ DAT with non-reactive eluate
↓

1 patient
  anti-Py' missed in pretransfusion testing
↓

58 patients with new alloantibody
↓

7 patients
  previously alloimmunized
↓

51 patients
  not previously alloimmunized
Autoantibody mimicking alloantibody

• Issitt PD et al. Studies of antibodies in the sera of patients who have made red cell antibodies. Transfusion 1996;36:481-486
  – 48/138 patient samples with panagglutinating autoantibodies contained 62 apparent alloantibodies
  – Alloabsorption with antigen negative cells indicated that 29 (47%) were partially absorbed autoantibodies that mimicked alloantibodies
  – Real alloimmunisation occurred in 23% of patients with panagglutinating autoantibodies

Hyperhaemolysis and bystander immune haemolysis

• SCD and Thal major patients have a high incidence of alloimmunisation and also autoimmunisation. Some have haemolytic crisis following transfusion with compatible rbcs
  – Hyperhaemolysis
    • Over active recognition by macrophages of antibody or complement coated cells in spleen and or liver
    • Defective erythropoiesis secondary to infection and systemic disease
  – Bystander immune haemolysis
    • Autoantibodies that mimic alloantibodies and cause red cell destruction by intra and/or extravascular mechanisms
    • Alloantibody interaction with donor rbc epitope leads to neoantigen producing and autoantibody.
Microchimerism

- Most donor lymphocytes eliminated in 3 days, some proliferate but are also eliminated in a few days
- Most transfusion based microchimerism is seen in patients receiving many units of blood due to trauma
- Only one donor results in the microchimera
- Incidence slightly less with leuco-depleted blood
- In materno-fetal situations microchimerism can be in both directions and in mothers can last many years
- Presence of donor lymphocytes: none, beneficial role in tissue repair, autoimmune disease/GVHD
- Age of blood transfused and time of transfusion following physiological challenge influence development

Epitope spreading

Foreign  Self

![Epitope spreading diagram]
Polyfunctional antibodies

Immune regulation

  - Reduced Treg activity in alloantibody responders
  - 50% of alloantibody responders had increased IL-4 expression on CD4+ cells
  - All patients had increased Th17 responses indicating an underlying inflammatory response
IL-17, Tfh and Th2 cells

- **Tfh (Tfh)**
  - Differentiation
  - Division
  - IL-4
  - Heavy chain class switching
  - IgM
  - IgG

- **Th2**
  - Innate IL-17
  - IL-17, IL-21
  - Pro-inflammatory, Th2 differentiation, neut recruit
  - Promote autoimmune responses
  - TGF-β
  - IL-6/IL-21

- **Th17**
  - TGF-β, IL-6/IL-21

- **Treg**
  - IL-17, IL-21
  - Pro-inflammatory, Th2 differentiation, neut recruit
  - Promote autoimmune responses
  - Down regulate T helper cell populations
  - CD4, CD25, FOXP3

- **Tnaive**
Conclusion

• Blood transfusion can result in the formation of alloantibodies and autoantibodies
• Autoantibody formation maybe benign, transient, mimic alloantibody specificity and in some cases cause destruction of the patients own red cells
• Autologous red cell destruction has been called reactive lysis, bystander immune haemolysis and hyperhaemolysis
• Microchimerism may play a part in apparent autoantibody formation
• Pro-inflammatory responses involving Treg downregulation and Th17 upregulation favour autoimmune responses

Thank you