Transfusion Related Immune Modulation (TRIM) Review

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Disclosures

• Nothing to report
Outline

• Immune Modulation
• Historical Background of TRIM
• Reported Effects on Innate and Adaptive Immune Systems
• “Storage” lesion
• Proposed Mechanisms
• Clinical Studies
• Future Directions
What is an Immune-Modulator?

• Definition: The adjustment of the immune response to a desired level, as in immunopotentiation, immunosuppression, or induction of immunological tolerance
Historical Background

1945: Medawar Demonstrates That Blood Shared Antigens with Other Tissues. He notes that some Transfused animals reject skin grafts From the blood donor as briskly As animals immunized to the donor By prior skin grafts.

1964: Halasz et al. Report that dogs Given donor blood with a renal graft Had prolonged survival
Improvement of Kidney-graft Survival with Increased Numbers of Blood Transfusions

Gerhard Opelz, M.D., and Paul F. Terris, M.D.

In a study of 1930 cadaveric-donor kidney transplants, we found a striking correlation of increased numbers of pretransplant blood transfusions with improved transplant survival (p<0.0001). Graft survival rate in recipients with >20 transfusions was 71±5 per cent at one year as compared with 62±5 per cent for recipients with no transfusions. At two years the survival rates were 65±5 per cent and 50±3 per cent (p<0.05). Fetal blood was less effective than nonfetal blood in producing this effect. In conclusion, we originally showed that patients who required kidney transplants without prior "immunizations" with blood transfusions had a lower transplant survival rate than patients who were pretreated with blood transfusions.1 This paradoxical finding has now been confirmed by 18 subsequent studies.2,3 Because these studies have emerged from so many diverse centers, it seems likely that this beneficial effect of transfusions is genuine. The important new question is how to use this effect to improve kidney transplant survival rates.2 We had previously suggested that the declining transplant survival rates in North America over the past few years could be attributed to the withholding of blood transfusions.4 With reinitiation of transfusions as a deliberate treatment, it will be important to know what dose schedules and timing would be optimal as well as what forms of blood transfusions would be most effective. There have been claims, for example, that a single transfusion is as effective as multiple ones and that transfusions at the time of transplantation only is as effective as transfusion before transplantation.5 As we demonstrate here, both these conclusions are in error, because transplant survival rates do not increase in proportion to the number of transfusions given before transplantation, and transfusions given at the time of transplantation have no statistically significant effect on graft outcome. The current analysis provides new data on a completely new set of patients studied during the past year.

Materials and Methods

Transplant centers participating with the University of California at Los Angeles (UCLA) transplant registry were asked in July, 1977 to supply transfusion data only on patients in whom an accurate transfusion history could be reliably documented. The centers were specifically requested to exclude patients who were not transfused, and the accuracy of records was particularly emphasized. As part of the number of unrecorded blood units was requested. Pretransplant transfusions were recorded separately from transfusions given during transplantation. The transfusions were grouped into three categories: whole blood, packed cell units containing washed, buffy-coat-free blood, and frozen cells. No distinction was made between different methods of preparing blood.

In his follow-up study he conducted a multicenter prospective RCT of potential candidates of cadaveric kidneys randomized to receive either ABT or no transfusions. At 1 year, the survival rate of transplanted kidneys was 90% in the ABT arm and 82% in those that didn’t receive ABT (p=0.02). This effect persisted at 5 year follow up and were evident even in the group that received only 1 unit of blood.

1972: Opelz et al. Conduct a large, retrospective Study to determine the exact effect of prior Transfusion on graft survival.
• This study led to a widespread practice of deliberately transfusing patients on transplant lists

• It came to a halt with the AIDS and Hep C epidemics
Mechanisms of transfusion-induced immunosuppression

M.E. Brunson and J.W. Alexander

In the 1980’s: Brunson and others begin using the term Transfusion Associated Immunosuppression to explain the effects seen in renal graft patients.

The recognition that prior blood transfusions decrease the rejection of organs is one of the most important discoveries in the history of transplantation. The current clinical question is whether transfusions might also retard immune responses to tumors or infections. In the 10 years since that possibility was suggested, numerous studies have correlated patients’ transfusion history to clinical outcomes of treatment for malignancy or postoperative infections. Most studies show that transfused patients have worse clinical outcomes. Although that finding is in no way proven causation, it certainly raises concerns.

Intravenously, most laboratory research has been directed toward finding ways to enhance the immunosuppressive effect of blood transfusion. This has happened because the transfusion effect is one of the most powerful influences on transplant graft survival. In the elucidation of blood transfusion’s prolongation of transplants, mechanisms have been demonstrated that suggest how immune responses to neoplasms or infections could be suppressed.

History of the Transfusion Effect

Study of the alteration of immune responses by blood transfusion began in 1945 when Medawar demonstrated that blood shared antigens with other tissues. He noted that some transfused animals rejected skin grafts from the blood donor as briskly as animals immunized to the donor by prior skin grafts. Clearly, this second-set phenomenon could occur only if some donor antigens were expressed on both blood and skin. This information strongly influenced the early years of human organ transplantation. Clinicians avoided giving transfusions to potential renal transplant recipients, reasoning that a blood transfusion shared antigens with a subsequent donor organ, the recipient might become immunized to those antigens and later reject the graft.

In retrospective, blood transfusion in Medawar’s model did not always shorten transplant survival. Furthermore, Halsey et al. reported in 1964 that dogs given donor blood had prolonged survival of renal grafts. Over the next 10 years, clinical and experimental evidence accumulated that blood transfusion might improve, rather than harm, cadaveric renal graft survival. In 1972 and 1973, Opelka et al. conducted a large, retrospective study to determine the exact effect of prior transfusion on graft survival. Contrary to contemporary expectations, a history of blood transfusion was associated with an increase in graft survival of as much as 20 percent. The greatest benefit occurred in patients receiving five or more transfusions prior to transplantation.

Clinical transplantation changed quickly after the discovery of the transfusion effect. The first deliberate preoperative treatment of kidney transplant recipients with donor-specific transfusions (DSTs) yielded approximately 30 percent better graft survival than that seen in graft recipients who had no DSTs. Unfortunately, some recipients became immunized to their designated organ donors by the DSTs and then had to wait for an acceptable cadaveric donor. Strategies evolved that minimized the risk of immunization, but they still do not completely prevent it. Another unresolved problem is that transplantation is postponed until the course of DSTs has been completed.

The transfusion effect has also been used for cadaveric transplants. During the 1970s, previously untransfused patients at an increasing number of centers were given several random-donor blood transfusions before being placed on the waiting list for transplantation.

Over the last decade, the benefit of transfusions has been steadily less demonstrable, mainly because of improved survival in nontransfused patients. One important reason for this overall improvement is the introduction of the powerful immunosuppressive drug, cyclosporin A (CyA). Although the use of CyA is rapidly changing immunosuppressive protocols in clinical transplantation, the deliberate pretransplant administration of either random-donor or donor-specific transfusions is still widespread.

Explanations for the Transfusion Effect

The associations between blood transfusion and differences in graft survival, postoperative infections, or
• Transfusion Associated Immune Suppression
• Transfusion Induced Immune Suppression
• Transfusion Related Immune Modulation
• A concept to potentially explain numerous clinical observations that suggest that RBC transfusion is associated with increased proinflammatory or immunosuppressive effects that may increase morbidity in at least some patient groups
Potentially Vulnerable Products and Cells Implicated

• RBCs
• Plasma
• Platelets
• Vascular Endothelial cells

All of these are highly responsive to inflammatory signals and when activated release significant quantities of potent bioactive mediators.
Other Considerations

Storage Lesion Effects?
Leukoreduction Effects?
### Effects of RBC storage lesion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Effect</th>
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<tbody>
<tr>
<td>pH, ATP</td>
<td>Echinocytosis</td>
</tr>
<tr>
<td>2,3 diphosphoglycerate (2,3 DPG)</td>
<td>Osmotic fragility</td>
</tr>
<tr>
<td>Glutathione</td>
<td>Mechanical fragility</td>
</tr>
<tr>
<td>NADH/NADPH</td>
<td>Vascular adhesion</td>
</tr>
<tr>
<td>Free Hb/heme/Fe^{2+}/Fe^{3+}</td>
<td>RBC microvesiculation</td>
</tr>
<tr>
<td>Extracellular K^+</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>Na^+ / K^+ pump paralysis</td>
<td>RBC aggregability</td>
</tr>
</tbody>
</table>

- **↑** Increase
- **↓** Decrease

### Documented Effects

- **Red Blood Cell**
  - Protein Kinase C
  - Reactive Oxygen Species (ROS)
  - Apoptosis

- **Neutrophil**
  - Leukotriene B_4
  - ROS
  - Pro-inflammatory cytokines
  - HMGB1 release
  - TLR4 activation

- **Macrophage**
  - ROS
  - Pro-inflammatory cytokines

- **Epithelial**
  - Free radical production
  - EGFR and MyD88 activation

- **Endothelial**
  - Cell adhesion molecule expression
  - Oxidized LDL deposition
  - Sensitization to oxidant challenge
  - Uncoupled eNOS
  - Direct incorporation through cell membrane

- **HEMOLYSIS**
  - Scavenging NO
  - Decreased NO bioavailability
  - Endothelial dysfunction
  - ROS, vasoconstriction

- **NO (nitric oxide)**
  - Direct oxidative hepatic and renal cytotoxicity
  - Release of IL-8 and IL-10

- **Free heme**
  - Neutrophil recruitment
  - ROS production
  - Systemic and local inflammation

- **Post-transfusion clearance**
  - Storage for up to 42 days
**“Two Insult” Model**

**HIT 1**
Patient’s Underlying Inflammation

Primes the Patient’s Immune Cells or Endothelium

**HIT 2**
Transfusion

Frank Inflammation Resulting in Full-Scale Activation
What are These Responses
4 Possible TRIM Mechanisms

- TRIM effect of ABT mediated by immunologically active allogeneic WBCS that down regulate the recipients immune function
- TRIM effect of ABT mediated by soluble biologic response modifiers released in a time-dependent manner from WBC granules or membranes into supernatant of stored RBCs
- TRIM effect of ABT mediated by soluble HLA peptides or other soluble mediators that circulate in allogeneic plasma
- Non-TRIM effect where ABT causes postoperative organ dysfunction predisposing to infection

Additional Mechanisms

• Cell-Associated changes on Innate Immunity
• Bioactive Soluble Factors that Influence Adaptive Immunity
• Microchimerism
White Blood Cells Are Likely The Primary Culprit in ABT

Leukocyte-containing RBCs contain viable and apoptotic WBCs, RBCs, Platelets and factors released during storage

WBCs, upon exposure to acidic conditions such as storage become activated and release cytokines

Cell Associated Mechanisms of TRIM include HLA class II-bearing donor dendritic APC associated with transfused allogeneic WBCs

White Blood Cells Are Likely The Primary Culprit in ABT

- WBC apoptosis begins immediately after blood withdrawal with granulocytes, then monocytes; while lymphocytes can remain viable for more than 25 days
- These apoptotic cells engage the phosphatidylserine/annexin V receptor on macrophages, inducing release of PG E-2 and TGF-β, suppressing macrophages and NK cells impairing APC capacity
Additional Effects on WBC from Storage

- Viable WBCs can act as responder cells or as stimulator cells inducing cellular immunity or antibody production in the recipient.
- After 3-5 days of storage a functional phosphorylation defect impairs protein synthesis of T cells upon signaling of the T-Cell Receptor and reduces the responder capacity of donor lymphocytes against recipient.
- After 10-14 days of storage, the capacity of donor APCs to stimulate recipient T-helper cell is abrogated by a reduction in co-stimulatory molecules.
Immunosuppressive effects of red blood cells on monocytes are related to both storage time and storage solution

Jennifer Muszynski, Jyotsna Nateri, Kathleen Nicol, Kristin Greathouse, Lisa Hanson, and Mark Hall

- In *in vitro* monocyte co-culture experiments, PRBC stored for 14 and 21 days were more immunosuppressive than 7-day old PRBC.
Bioactive Soluble Factors

- Soluble factors accumulate during storage in the supernatant and include elastase, myeloperoxidase, histamine, soluble HLA, PAI-1, soluble FAS-ligand, TGF-β1, and pro-inflammatory cytokines IL-1β, IL-6, and IL-8 inhibit neutrophil function
Microchimerisms

- When donor and recipient HLA compatibility is such that there is a persistence of small numbers of donor lymphocytes and APCs in circulation or organs of the ABT recipient.
- This may downregulate the immune response to release IL 4 and 10, transforms TGF-β from host TH2 cells inhibiting production of TH1 cells.
A Word About NTBI, PLI, Free Heme

• As NTBI, PLI, and Free heme increase exponentially after transfusion with longer, stored blood, Hod/Spitalnik and others have demonstrated in murine models that these increases increase bacterial growth, inhibition of macrophage activation, alter proliferation and activation of T, B, or NK cells, decreases NADPH-dependent oxidative burst, and increases in IL-4, 8, and 10.

Blood 2010;115-4284-4292.
Summary

Table 1  Postulated mechanisms of the TRIM effect.

- Clonal deletion of specific lines of immune cells
- Induction of suppressor T cells
- Production of anti-idiotypic antibodies
- Suppression of natural killer (NK) cell activity
- Polarization of the immune system to the T-helper type 2 responses, with suppression of T-helper type 1 responses
- Selection of non-responder type immune cells
- Mixed microchimerism
- Induction of apoptosis, resulting in the death of specific types of immune competent cells
- Accumulation in the supernatant of stored components of soluble molecules (e.g., histamine, eosinophil cationic protein, eosinophil protein X) that inhibit neutrophil function
- Accumulation in the supernatant of stored components of soluble molecules (i.e. soluble Fas ligand or soluble HLA class I molecules) that inhibit the immune response
What Does This All Mean Clinically?
## Clinical relevance of TRIM effect

<table>
<thead>
<tr>
<th>Benefits</th>
<th>Risks</th>
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<tr>
<td>Improved renal allograft survival after ABT</td>
<td>Possible increased cancer recurrence</td>
</tr>
<tr>
<td>Beneficial in treatment of recurrent spontaneous abortion</td>
<td>Possible increased perioperative infection</td>
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<td></td>
<td>Increased short-term mortality in cardiac surgery setting</td>
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On Postoperative Infection

• Over 20 RCTs and 40 observational studies conducted investigating the possible association between perioperative ABT and postoperative infection

• No effect to a 7.3 fold increase in infections in ABT patients

• Meta-analysis by Vamvakas et al. found no significant association between the outcomes of patients that received ABTs vs. those that did not
What About Using WBC reduction?

- Prestorage leukoreduction leaves a leukocyte count of $<5 \times 10^6$
- This nearly eliminates CMV, EBV, reduces HLA allo-immunization, bacterial/parasite contamination
- Sparrow et al. has demonstrated that prestorage reduction appears to mitigate cytokine release by allogeneic neutrophils although supernatant does still induce regulatory T cells
- In clinical studies, only postoperative cardiac patients have demonstrated a benefit in reduction in postoperative infection

Blood Reviews, Volume 21, Issue 6, 2007, 327 - 348
Randomized Controlled Trials Investigating the Association of WBC Containing ABT with Postoperative Infection

P = NS
Randomized Controlled Trials Investigating the Association of WBC Containing ABT with Short Term Mortality

Blood Reviews, Volume 21, Issue 6, 2007, 327 - 348
Randomized Controlled Trials Investigating the Association of WBC Containing ABT with Mortality from All-Causes

Blood Reviews, Volume 21, Issue 6, 2007, 327 - 348
Longer RBC Storage Duration Is Associated With Increased Postoperative Infections in Pediatric Cardiac Surgery

• Cholette et al. studied 128 children undergoing cardiac repair or palliation with CPB to assess outcomes including postoperative infections. This was part of another RCT examining washed vs. non-washed RBCs

• Outcome: The postoperative infection rate was significantly higher in children receiving the oldest blood (25-38 d) compared with those receiving the freshest RBCs (7-15 d) (34% vs 7%; p = 0.004)

• Subgroup analysis of subjects receiving only 1-2 RBC transfusions on the day of surgery (n = 74) also demonstrates a greater prevalence of infections in subjects receiving the oldest RBC units (0/33 [0%] with 7- to 15-day storage; 1/21 [5%] with 16- to 24-day storage; and 4/20 [20%] with 25- to 38-day storage; p = 0.01)
Methodological problems in the study of TRIM

Numerous observational studies, insufficient prospective double-blinded RCTs
Likelihood of confounding factors, i.e. patients receiving blood transfusions differ from those not receiving transfusions; to what extent are these adjusted for?
Difficulty in performing meta-analysis when studies insufficiently homogeneous
Widespread introduction of universal WBC reduction has decreased the opportunity to perform RCTs of WBC reduced vs non-WBC reduced ABTs
A relatively small TRIM effect may not be detected consistently, particularly in small studies

Potentially Vulnerable Populations

- Postoperative
- Trauma
- Sepsis
- Cardiac Surgery with CPB
- Transplantation
- Induced Immunosuppression
- Pediatrics
Anti-inflammatory Cytokines Suppress Or Stimulate APCs Skew TH1, TH2, TREGS

Sepsis
Interact with TLRs On Macrophages
Release Hormones, Cytokines, Chemokines, Coagulation and complement systems are activated

=Blood Transfusions

What Do We Study

• The Products
  Collection, Storage, Cells vs. Supernatant

• The Host Response
  Different in Differing Patient Populations

• The Host Response
  Different at different times in a single patient’s course?
Conclusions

Blood Reviews, Volume 21, Issue 6, 2007, 327 - 348
Conclusions

• TRIM is a real biologic phenomenon, resulting in at least one established beneficial clinical effect in humans (augmentation of renal allograft survival), however, the existence of the various deleterious clinical TRIM effects has not yet been confirmed by sufficiently powered RCTs.

• The only clinical situation where the findings of RCTs of adverse TRIM effects have been consistent is in cardiac surgery patients. In that setting, the use of WBC-reduced allogeneic RBCs has been shown to reduce the short-term (up to 3-month post transfusion) mortality from all causes.
Conclusions

• Pre-clinical and clinical studies are urgently needed to determine the mechanism(s) of the association between non-WBC-reduced allogeneic blood transfusion related all-cause mortality in cardiac surgery.

• Pre-clinical and clinical studies are needed to elucidate the mechanism(s) and clinical significance of the “pro-inflammatory” effect(s) of non-WBC-reduced allogeneic blood transfusions.

• Adequately powered RCTs are required to elucidate the existence (or not) of clinically relevant transfusion-related immunomodulation (TRIM).

• Pre-clinical and clinical studies are needed to elucidate the mechanisms of the other postulated immunomodulatory effects of allogeneic blood transfusions.
Where Do We Go From Here?

• What are the Burning Questions?
• What are the Best Initial Models?
• Who are the Best Patients to Study?