CHAPTER 62

Immunomodulatory and pro-inflammatory effects of allogeneic blood transfusion

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Introduction

The question we confront is whether observations of immunologic consequences of allogeneic blood transfusion (ABT) represent only laboratory curiosities or clinically relevant alterations in the recipient’s immune function—the so-called immunomodulatory effect of ABT.1 The constellation of all such ABT-associated laboratory and clinical findings is known as transfusion-related immunomodulation (TRIM). Initially, the term TRIM encompassed effects attributable to ABT by means of immunologic mechanisms only; however, more recently, the term has been used more broadly to encompass additional effects that could be related to ABT by means of both immunomodulatory and pro-inflammatory rather than only immunomodulatory mechanisms.2

ABT may either cause alloimmunization or induce tolerance. ABT introduces a multitude of foreign antigens into the recipient, including human leukocyte antigen cell surface receptor (HLA-DR) antigens found on the donor’s dendritic antigen-presenting cells (APCs). The presence or absence of autologous HLA-DR antigens on the donor’s white blood cells (WBCs) plays a decisive role in whether alloimmunization or immune suppression will ensue following ABT.3 Transfusions sharing at least one HLA-DR antigen with the recipient will induce tolerance, whereas fully HLA-DR-mismatched transfusions lead to alloimmunization.4 In addition to the degree of HLA-DR compatibility between donor and recipient, the immunogenicity of cellular or soluble HLA antigens found in transfused blood components depends on the viability of the donor dendritic APCs and the presence of the required co-stimulatory signals for the presentation of the donor antigens to the recipient’s T cells. Nonviable APCs and/or absence of the requisite co-stimulatory signals result in T-cell unresponsiveness.5–7 Thus, when a multitude of antigens is introduced into the host by ABT, the host response to some of these antigens is often decreased, and immune tolerance (or TRIM) ensues.8 Several immune-function alterations have been documented in association with ABT (Table 62.1).9,10

All these ABT-related laboratory immune alterations could potentially be associated with clinical effects. Evidence from a variety of sources indicates that ABT enhances the survival of renal allografts.11 In addition, other possible effects are increase of the recurrence rate of resected malignancies and the incidence of postoperative bacterial infections, reduction of the recurrence rate of Crohn’s disease12 and the risk of fetal loss in women with recurrent spontaneous abortions (RSAs), activation of infections with cytomegalovirus (CMV) or human immunodeficiency virus (HIV), and increased short-term (up to 3 months post transfusion) mortality from all causes.13,14

Different biologic mechanisms may be involved in each of these purported clinical manifestations of TRIM, and the clinical evidence supporting each of the aforementioned hypotheses must be examined on its own merits.13,15 The specific constituent(s) of allogeneic blood that mediate(s) the TRIM effect(s) also remain(s) unknown, and published literature has suggested that TRIM effects may be mediated by one or more of the following: (1) soluble HLA class I peptides that circulate in allogeneic plasma; (2) soluble biologic response modifiers released in a time-dependent manner from WBC granules or membranes into the supernatant fluid of red blood cell (RBC) or platelet concentrates during storage; and/or (3) allogeneic mononuclear cells (Figure 62.1).15

Beneficial clinical TRIM effects

Enhanced survival of renal allografts

The only clearly established TRIM effect is beneficial: the enhanced survival of renal allografts in patients who have received pretransplant ABT.11,17 In both observational studies and randomized controlled trials (RCTs), patients transfused with allogeneic blood have been shown to have a significantly better renal-allograft survival than untransfused patients, regardless of the number of HLA-A, HLA-B, and HLA-DR locus mismatches between recipient and donor.11,18 This is true also when there is a common HLA haplotype, or shared HLA-B and HLA-DR antigens between donor and recipient.19 The TRIM effect has further been reported to be associated with allografts between HLA-identical siblings.20

The ABT-related enhancement of renal-allograft survival has been confirmed by animal data and clinical experience worldwide.18 In the past, it was a standard policy in many renal-transplant units to deliberately expose patients on transplant waiting lists to one or more allogeneic RBC transfusions. Subsequently, the beneficial effect of pretransplant ABT was thought to be less important with the advent of cyclosporine and other potent agents.
immunosuppressive drugs, and, as a consequence, many centers discontinued its use.

However, a multicenter observational study, reporting on 58,036 renal allografts from cadaveric donors after the advent of cyclosporine, indicated that patients who had received ABT were still more likely to have a successful renal allograft than those who had not. This study reported that the one-year renal-allograft survival of patients receiving pretransplant ABT was 3–5% better than that of those who did not receive ABT. Similar results were also reported for patients who received renal allografts from living-related donors. The beneficial effect of pretransplant ABT in the outcome of cadaveric renal allografts was confirmed by a RCT conducted at 14 transplant centers. Patients were randomly assigned to receive either three pretransplant, non-WBC-reduced RBC transfusions or no ABT. The renal-allograft survival was significantly higher in the 205 transfused patients than in the 218 untransfused subjects (90% vs. 82% at 1 year, $p = 0.02$; 79% vs. 70% at 5 years, $p = 0.025$). The beneficial effect of ABT was found to be independent of age, gender, underlying disease, prophylaxis with lymphocyte antibodies, or the presence of preformed lymphocytotoxins.

There have been two more RCTs that compared types of pretransplant ABTs given to prolong graft survival. Both studies were small, enrolling 52 and 144 patients, respectively. The first RCT compared non-WBC-reduced and WBC-reduced RBCs and found no difference in graft survival. However, the WBC-reduced RBCs administered in this 1985 RCT did not meet the current European WBC reduction standard ($<10^6$ WBCs/unit), and all transfused RBC components may have been equally effective in mediating the ABT effect. The other RCT compared recipients of one HLA-DR-mismatched ABT, one HLA-DR-matched ABT, and no ABT. There was no difference in graft survival at one year or five years. The risk of acute rejection in patients who had received an HLA-DR-shared ABT was lower than that observed in the other two groups (19% vs. 33%), but this difference did not attain statistical significance in this small RCT. The three available RCTs have employed different study designs and have addressed different clinical questions, so that the integration of their results in a meta-analysis is not possible owing to the clinical heterogeneity of the studies.

In observational studies, recipients of non-WBC-reduced whole blood or RBCs have had better one-year cadaveric-allograft survival than patients given WBC-reduced blood components such as frozen-thawed-deglycerolized RBCs. Such data indicate that allogenic WBCs are involved in eliciting this beneficial TRIM effect.

However, the mechanism(s) involved in the ABT-related enhancement of renal allograft survival remain(s) to be elucidated. An experimental-animal model has suggested that the beneficial effect of donor-specific ABT might be related to the type of transplanted organ. Whereas ABT appears to lead to permanent acceptance of all

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**Table 62.1** Documented immune function alterations in association with ABT

<table>
<thead>
<tr>
<th>Effect</th>
<th>Purportedly caused by</th>
<th>Prevented by</th>
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<tbody>
<tr>
<td>Decreased T-helper (CD4) cell count</td>
<td>Increased recurrence rate of resected malignancies</td>
<td>1. Autologous transfusion</td>
</tr>
<tr>
<td>Decreased helper/suppressor (CD4/CD8) T-lymphocyte ratio</td>
<td>Increased incidence of postoperative bacterial infections</td>
<td>1. Fresh autologous blood obtained by ANH, IBR, or PBR</td>
</tr>
<tr>
<td>Decreased lymphocyte response to mitogens</td>
<td>Activation of endogenous CMV or HIV infections</td>
<td>2. Prestorage WBC reduction</td>
</tr>
<tr>
<td>Reduction in delayed type hypersensitivity</td>
<td>Increased short-term (up to 3-month) mortality</td>
<td>1. Autologous transfusion</td>
</tr>
<tr>
<td>Decreased natural-killer (NK) cell function</td>
<td></td>
<td>2. Prestorage WBC reduction</td>
</tr>
<tr>
<td>Hypergammaglobulinemia</td>
<td></td>
<td>3. Poststorage WBC reduction</td>
</tr>
<tr>
<td>Decreased cytokine (IL2, interferon-γ) production</td>
<td></td>
<td></td>
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<tr>
<td>Suppression of lymphocyte blastogenesis</td>
<td></td>
<td></td>
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<tr>
<td>Decreased lymphocyte response to antigens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased natural-killer (NK) cell function</td>
<td></td>
<td></td>
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<tr>
<td>Increased production of anti-idiotypic antibodies</td>
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</table>

**Figure 62.1** TRIM effects, postulated mediators of TRIM, and preventive strategies that could be effective if the TRIM effects were mediated by each corresponding mediator. The purported deleterious TRIM effects are mediated by an unknown constituent(s) of allogenic blood that may (or may not) include one (or more) of the mediators shown in the figure. Stored autologous blood, obtained by preoperative autologous blood donation (PABD), is replete with WBC-derived soluble mediators because both autologous and allogenic WBCs deteriorate equally during storage, releasing such mediators. Fresh autologous blood, transfused within hours of collection and processing, is free of WBC-derived soluble mediators and can be procured by acute normovolemic hemodilution (ANH), intraoperative blood recovery (IBR), or postoperative blood recovery (PBR). WBC-reduction filters do not retain WBC-derived soluble mediators, accounting for the difference between prestorage and poststorage WBC reduction in abrogating the TRIM effects mediated by such mediators. Source: Vamvakas (2006). Reproduced with permission of American Society for Clinical Pathology.
renal allografts, this benefit was not observed with pancreas, skin, or heart allografts. ABT administered during the actual operation for renal transplantation has not been shown to affect subsequent allograft survival. In observational studies, patients who receive more than 10 RBCs have a better one-year allograft survival than patients who receive only one or two RBC units. However, patients who receive more than 10 RBCs appear to have a poorer overall allograft survival than those who receive fewer than 10 RBC units.

Such data suggest that multitransfused patients often develop cytotoxic antibodies and are thus at greater risk for earlier and more severe allograft-rejection episodes. Along these lines, Solheim has discerned another potential benefit from pretransplant ABTs, which is especially relevant in settings where there is a shortage of organs for transplantation. When several prospective recipients on a renal-transplant list produce crossmatch-negative results with an available organ, pretransplant ABTs could help identify high-responder patients (i.e., patients most likely to form cytotoxic antibodies in response to pretransplant ABTs, and also most likely to reject a transplanted kidney because of formation of cytotoxic antibodies after a negative crossmatch). Transplant surgeons could thus channel the scarce organ away from such patients, and give it to a patient in whom it is most likely to survive.

A recent Agency for Healthcare Research and Quality (AHRQ) review of all available clinical evidence suggested that pretransplant ABT has a neutral to beneficial effect on graft rejection, graft survival, and patient survival compared with no ABT. However, such benefits were reported mostly in studies conducted before the introduction of modern immunosuppressive drugs and solid-phase technology to detect formation of cytotoxic antibodies. In addition, the strength of the evidence was low.

Three other recent reviews of the clinical literature concluded that pretransplant ABTs place patients at increased risk of forming cytotoxic antibodies, and that the ensuing HLA alloimmunization reduces renal allograft survival and increases wait time for transplantation. By searching the MEDLINE, Embase, and Cochrane Library datasets for English-language publications between January 1984 and March 2011, the latest analysis captured 180 studies and data from publicly available registries. The authors noted that implementation of universal WBC reduction had not decreased HLA alloimmunization in patients receiving renal allografts to any significant extent. Although a recent study again reported a beneficial effect of pretransplant ABT with current immunosuppression protocols such as cyclosporine, most current evidence appears to indicate that improvements in graft survival comparable to those previously ascribed to the beneficial "ABT effect" are now attainable without pretransplant ABTs. For this reason, the graft outcome risk–benefit ratio has now become too high to justify consideration of pretransplant ABTs when these can be avoided.

In summary, the beneficial ABT effect in renal transplantation is established, and it used to be clinically relevant before the advent of cyclosporine and similar immunosuppression regimens. Before 1984, the demonstration of this beneficial ABT effect had led to the consideration of pretransplant ABT(s) as a strategy to improve renal allograft survival in transplant recipients. However, with the rapid improvement in peri-transplant immunosuppression therapy, the additional effect of ABT became marginal. For this reason, the relevance of such pretransplant ABT protocols in clinical practice diminished because of the improvements in HLA technology and the remarkable advances in targeted and safe immunosuppression. Alloimmunization to HLA antigens can occur despite the use of WBC-reduced blood components. Therefore, in the modern era, the use of pretransplant ABTs should be avoided whenever possible. When ABT is necessary in patients awaiting renal transplantation, WBC-reduced blood components must be given to reduce the risks of HLA alloimmunization and CMV transmission.

### Reduced risk of RSAs

Transfusion of allogeneic WBCs has been proposed as a form of immunotherapy for the prevention of RSAs. In this setting, the fetus represents a semi-allogeneic graft to its mother, and maintenance of a pregnancy depends on immunologic equilibrium between the implanted fetus and the maternal immune response to the fetus. When the genetic parents share HLA antigens, this balance may be altered and maternal blocking antibodies may not be formed, predisposing the pregnant woman to RSAs. Different transfusion protocols of allogeneic WBCs have been used at various centers to reduce the risk of RSAs, employing WBCs obtained from either sexual partners or third-party donors. Such WBCs have been administered intravenously, intracutaneously, or as an intradermal injection of mononuclear cells obtained by gradient separation. WBC-containing blood products have included pooled buffy coats, single-donor buffy coats, or RBC suspensions containing WBCs.

The American Society for Reproductive Immunology (ASRI) conducted a worldwide collaborative individual-patient-data (IPD) meta-analysis to examine the efficacy of immunotherapy with allogeneic WBCs in patients with a history of RSAs and concluded that such treatment was effective. The effect was small, with only 8–10% of affected women benefiting from the treatment. In contrast to the ASRI data, a recent multicenter RCT that enrolled 183 women with a history of three or more spontaneous abortions reported a nonsignificant decrease in live-birth rate in patients randomly assigned to immunotherapy as compared with controls (36% vs. 48%). However, the size of this RCT was likely inadequate to establish a clinically relevant treatment effect. Thus, the efficacy of transfusions of allogeneic WBCs for the treatment of patients with RSAs remains to be established.

### Reducted risk of recurrence of Crohn’s disease

Several observational studies have examined whether postoperative recurrence in patients with Crohn’s disease (which is immune-mediated) can be reduced by the perioperative administration of ABT. Pooled data from the available studies suggest that the recurrence rate in transfused and untransfused patients is similar: 37.5% versus 40.5%. However, such integration of the reported data may be inappropriate, because the available studies are observational and medically heterogeneous (i.e., using different follow-up periods and surgical interventions). Nonetheless, an IPD meta-analysis of 622 patients with Crohn’s disease found no effect of perioperative ABT on subsequent need for surgical intervention, independent of age, gender, disease location, or extent of the resection. Because many factors affect the risk of recurrence in patients with Crohn’s disease, a large RCT is needed to clarify the role of ABT, if any, in modulating Crohn’s disease activity in patients with this disorder.

### Deleterious clinical TRIM effects

#### Increased recurrence of resected malignancies

If ABT has a beneficial effect in renal transplantation, where immunosuppression is beneficial because it may prevent allograft rejection, ABT could also have deleterious effects in situations...
where impairment of the recipient’s immune function can be detrimental. In 1981, eight years after the first report of the beneficial effect of pretransplant ABT on renal allograft survival,11 Gant45 voiced his concern that perioperative ABT for curative resection of a malignancy might provoke recurrence of cancer. Thus, if the host’s immune response to a tumor contributes to controlling the tumor’s growth, the impairment of the host’s immunity, due to ABT, would impair this defense mechanism and facilitate tumor growth.

More than 100 observational studies of ABT and cancer recurrence have been reported,46 and their unadjusted results (i.e., their findings before adjustment for the effects of confounding factors) were subjected to five meta-analyses.46–50 When the available results were integrated for seven cancer sites, there was agreement between the five overviews on the magnitude and statistical significance of the risk of cancer recurrence, death due to cancer recurrence, or overall mortality in transfused compared with untransfused patients. A statistically significant adverse clinical outcome was found among transfused (compared with untransfused) patients for all cancer sites evaluated except for cervix.46 Brand and Houbiers46 and Vamvakas48 ascribed this summary TRIM effect (detected across the unadjusted analyses of the observational studies) to the effects of confounding factors, whereas recent meta-analyses49,50 portrayed the summary TRIM effect as a real deleterious effect of ABT.

Not finding an adverse effect of ABT when the unadjusted results of the observational studies of cervical cancer are integrated is puzzling.46 Virus-associated cancers (e.g., cervical cancer) are considered immunogenic, and, should ABT exercise a TRIM effect, the TRIM effect should be apparent specifically in cervical cancer (as opposed to cancers from other investigated sites). By the time of the meta-analysis of Brand and Houbiers,46 the association between ABT and cervical cancer recurrence had been investigated in six observational studies that had included more than 1000 women. Although the low overall recurrence rate in the six studies precluded a definitive conclusion, no adverse ABT effect was detected across the six studies.46 These results were possibly due to the fact that, in the development of cervical intraepithelial neoplasia, the expression of HLA class I molecules on malignant cells is specifically downregulated. Human papillomavirus–specific cytotoxic cells (whose function might potentially be suppressed by ABT) may thus be effective against cervical cancer cells because of this reduction in class I HLA molecule expression.46

Observational studies reporting a significant ABT effect on cancer recurrence continue to be reported,53 including current reports of a dose-dependent ABT effect mediated by WBC-reduced RBCs.53 The demonstration of a dose-dependent ABT effect in patients receiving WBC-reduced RBCs versus no ABT in the current era52 parallels the demonstration of a dose-dependent ABT effect in patients receiving non-WBC-reduced RBCs versus no ABT in the numerous (now historical) observational analyses of the 1980s and 1990s.46–50 Although they do not rule out the existence of an adverse TRIM effect, the current observations52 link the dose-dependent ABT effect to illness severity necessitating ABT, rather than to a TRIM effect promoting cancer recurrence and mediated by allogeneic WBCs. Similarly, the ABT effect attributed to non-WBC-reduced RBCs in the numerous (now historical) observational analyses of the 1980s and 1990s46–50 was not confirmed by the available RCTs of perioperative ABT and cancer recurrence, which are discussed in the “TRIM Effects Mediated by Allogeneic Mononuclear Cells” section.

**Increased risk of postoperative bacterial infection**

Approximately 40 observational studies,53–58 which had compared the risk of postoperative bacterial infection between transfused and untransfused patients undergoing gastrointestinal surgery, orthopedic operations, cardiac surgery, or various other surgical procedures, seemed to indicate that patients receiving perioperative ABT (compared with those not receiving ABT) had a higher risk of developing postoperative bacterial infection.53 The studies also indicated that patients receiving transfusion generally differed from those not receiving transfusion in several prognostic factors that predisposed to adverse clinical outcomes.54 Based on these two sets of observations, some authors concluded that ABT has a direct deleterious effect on the recipient, causing an increased risk of postoperative bacterial infection.53 Other investigators concluded that clinical need for ABT can be a surrogate marker for a variety of adverse prognostic factors and that the other variables that generated the need for ABT in the published studies also determined the subsequent clinical outcome.54

Currently, the controversy over TRIM is focused on differing interpretations55–58 of the findings of the available RCTs of perioperative ABT and postoperative infection. Hitherto, there have been 22 RCTs59–70 that compared the risk of postoperative infection and/or mortality between patients randomly assigned to receive non-WBC-reduced allogeneic versus autologous or WBC-reduced allogeneic RBCs. Three of these RCTs59–60,62,64,68–70,73,76–77 also compared the risk of cancer recurrence between the two randomization arms. Nineteen RCTs were conducted in the perioperative setting; three enrolled HIV-seropositive patients,72 all hospitalized patients,75 or trauma patients.80

Based on an integration of the results of all nine RCTs published or reported through 200559,62,64,68–70,73,76–77 and comparing the risk of postoperative infection between patients randomly assigned to receive non-WBC-reduced versus WBC-reduced ABT in the event that they needed perioperative transfusion, two meta-analyses55,66 concluded that non-WBC-reduced ABT is associated with postoperative infection. In contrast, a third meta-analysis57 that integrated the findings of all 12 RCTs published or reported through 200559,62,64,68–70,73,76–77 found no association between non-WBC-reduced ABT and postoperative infection. Similarly, no association between ABT and postoperative infection was detected when the findings of RCTs59,60,61,63,66,74 comparing recipients of allogeneic and autologous RBCs were integrated.52

Two principal reasons for the disagreements between the three meta-analyses55–57 are (1) the inclusion in the analysis of all 12 RCTs available today versus the nine initially published RCTs; and (2) the integration (or not) of the results of all 12 (or nine) RCTs despite extreme medical heterogeneity. Medical heterogeneity included such factors as the RBC product transfused to the non-WBC-reduced arm (i.e., non-buffy-coat-reduced versus buffy-coat-reduced allogeneic RBCs or whole blood), the RBC product transfused to the WBC-reduced arm (i.e., WBC-reduced RBCs or whole blood filtered before or after storage), the transfusion dose, the surgical setting (gastrointestinal, cardiac, or other), the types of postoperative infections evaluated, the criteria for diagnosing postoperative infection, and the frequency of postoperative infection recorded in each study.58

One would expect (1) more of a TRIM effect in association with the transfusion of non-buffy-coat-reduced (compared with buffy-coat-reduced) allogeneic RBCs, because the buffy-coat-reduced RBCs used in Europe contain only about one-third of the donor
WBCs found in the non-buffy-coat-reduced RBCs used in North America; (2) a greater reduction of a TRIM effect(s) attributable to the transfusion of prestorage- (compared with poststorage-) filtered, WBC-reduced allogeneic RBCs, because of the removal of WBC-derived soluble mediators through prestorage (but not poststorage) filtration (Figure 62.1); and (3) more of a TRIM effect in cardiac (compared with other) surgical settings, because in cardiac surgery allogeneic mononuclear cells and/or WBC-derived soluble mediators might serve as a second inflammatory insult that compounds the diffuse inflammatory response to the extracorporeal circuit.53

Therefore, one would not expect all 12 RCTs available today (or all nine initially published RCTs) to have targeted the same TRIM effect. In contrast, depending on their design, these 12 (or 9) RCTs probably targeted TRIM effects that varied in magnitude and/or nature, making the integration of all 12 (or all 9) RCTs by a meta-analysis inappropriate. For example, RCTs administering RBCs filtered before storage to the WBC-reduced arm62,68,70,73,76–80 investigated TRIM effects mediated by both WBC-derived soluble mediators and allogeneic mononuclear cells (Figure 62.1), whereas RCTs transfusing RBCs filtered after storage to the WBC-reduced arm59,64,68,69 investigated only TRIM effects mediated by allogeneic mononuclear cells. Meta-analyses should integrate only results from subsets of RCTs that are medically sufficiently homogeneous to justify the assumption that all combined studies have targeted a TRIM effect that is biologically similar.58 Results from meta-analyses of such homogeneous subsets of RCTs are presented in the “TRIM Effects Mediated by Allogeneic Mononuclear Cells” section. Blumberg et al.56 had earlier attributed the disagreements between the three meta-analyses55–57 to the reliance on the intention-to-treat principle in the meta-analysis that did not detect an adverse TRIM effect of ABT57 versus the use of results from “as-treated” analyses in the two meta-analyses that reported a deleterious TRIM effect.55,58 Intention-to-treat analyses often have reduced statistical power to detect a treatment effect compared with “as-treated” analyses.56 However, both intention-to-treat and “as-treated” analyses demonstrated a deleterious TRIM effect when the analysis integrated the findings of all nine initially published RCTs; whereas neither the intention-to-treat nor the “as-treated” analysis showed an association between non-WBC-reduced ABT and postoperative infection when the analysis integrated the results of all 12 RCTs available today (Table 62.2).58

### Increased risk of short-term (up to 3 months posttransfusion) mortality

An association between non-WBC-reduced ABT and short-term (up to 3 months posttransfusion) mortality from all causes was described in the RCT of van de Watering et al.68 This RCT had been designed to investigate an association between non-WBC-reduced ABT and postoperative infection but instead observed an association between non-WBC-reduced ABT and mortality. The association between ABT and mortality was reported as a data-derived hypothesis,68 and the authors postulated that non-WBC-reduced ABT may predispose to multiple-organ failure (MOF), which might predispose to mortality. These investigators undertook another RCT that confirmed the association between ABT and mortality but did not find an association between non-WBC-reduced ABT and increased MOF.73

Several preclinical84–88 and clinical83,89–102 observations have supported the hypothesis that ABT in general, and non-WBC-reduced ABT in particular, may be associated with MOF. The mechanisms underlying the development of MOF are unclear, but most evidence suggests that tissue injury is mediated by reactive oxygen species and proteolytic enzymes released from activated neutrophils.100–102 Silliman et al.84 proposed that ABT may exercise a neutrophil-priming effect mediated by bioactive lipids that accumulate during storage. They postulated that rapidly deteriorating WBCs in stored RBCs release cytotoxic enzymes that may act on fragmented RBC membranes to produce mediators that are responsible for neutrophil priming and endothelial-cell activation (Figure 62.2).

These investigators84–86 demonstrated that plasma obtained from stored RBCs primes neutrophils for superoxide production and enhanced cytotoxicity, and also activates pulmonary endothelial cells in a dose- and age-dependent fashion. The length of RBC storage was important in these studies, because no evidence of neutrophil priming was obtained when plasma stored for short periods was used. Silliman et al.87 also showed that lipids from the plasma supernatant of RBCs stored for 42 days cause acute lung injury in isolated pulmonary models. Similarly, Chin-Yee et al. reported that plasma supernatant from stored RBCs activates neutrophils.88 In that study, WBC reduction of the RBC units abrogated the effect.88

Based on this observation that ascribes a neutrophil-priming effect to ABT (Figure 62.2), it is possible that the reported68,73

### Table 62.2 Meta-analyses of RCTs of non-WBC-reduced ABT and postoperative infection: impact of the method of analysis and the number of RCTs included in the analysis

<table>
<thead>
<tr>
<th>Number of RCTs Included in the Analysis</th>
<th>Method of Analysis</th>
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<tbody>
<tr>
<td></td>
<td>Intention-to-Treat*</td>
</tr>
<tr>
<td></td>
<td>Number of Patients</td>
</tr>
<tr>
<td></td>
<td>Analyzed</td>
</tr>
<tr>
<td>9 RCTs62,64,68–70,73,76–77 published or reported through 200255,56</td>
<td>5017</td>
</tr>
<tr>
<td>12 RCTs59,62,64,68–70,73,76–80 published or reported through 200557</td>
<td>6290</td>
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*The intention-to-treat analyses included all patients randomly assigned preoperatively to receive non-WBC-reduced or WBC-reduced ABT in the event that they needed perioperative transfusion. The “as-treated” analyses retained only those patients from each randomization arm who ended up receiving transfusion during or after surgery.

1Integration of all nine (or all 12) RCTs, as shown in this table, is inappropriate because of the extreme medical heterogeneity of the studies (see text). Therefore, readers should resist the temptation to assign a medical or biological meaning to the figures presented in the table. Instead, readers are referred to Figures 62.4 and 62.5, which depict the results obtained when medically homogeneous subsets of these RCTs59,62,64,68–70,73,76–80 were integrated.

The percentage of all randomized patients that was included in the “as-treated” analyses is given within parentheses.

*Statistically significant adverse TRIM effect (p<0.05).

association between ABT and short-term mortality could in fact reflect a “pro-inflammatory” rather than an “immunomodulatory” effect of ABT covered under the general concept of adverse TRIM effects, which may include effects attributable to ABT by either immunomodulatory or pro-inflammatory mechanisms, or by a combination of these mechanisms.

In the study of Johnston et al., patients receiving autologous RBCs had a significantly higher risk of MOF than recipients of polymerized hemoglobin. Neutrophils obtained from recipients of RBCs demonstrated priming, as evidenced by increased beta-2 integrin expression, superoxide production, and elastase release. Neutrophils obtained from recipients of polymerized hemoglobin showed no evidence of priming. Studies investigating the benefits obtained from placing a WBC reduction filter in the arterial line of the cardiopulmonary bypass circuit suggested that non-WBC-reduced ABT may provoke cardiac and/or pulmonary failure. Furthermore, associations between ABT and prolonged mechanical ventilation or MOF were reported by some, but not all, observational studies.

Eleven RCTs, comparing recipients of non-WBC-reduced versus WBC-reduced autologous RBCs, and reporting on cancer recurrence, postoperative infection, or mortality as the primary outcome, have presented information on short-term (up to three months posttransfusion) mortality from all causes. Because WBC reduction filters do not retain soluble mediators, if ABT exercised the described WBC-dependent neutrophil-priming effect that is mediated by bioactive lipids that accumulate during storage (Figure 62.2), allogeneic RBCs that are WBC-reduced before storage should abrogate this effect; but allogeneic RBCs that are WBC-reduced after storage should confer no benefit. Despite this theoretical prediction, no increase in mortality in association with non-WBC-reduced ABT was detected either across the subset of RCTs transfusing RBCs filtered before storage to the WBC-reduced arm, or across the subset of RCTs transfusing RBCs filtered after storage to the WBC-reduced arm.

However, across five RCTs conducted in cardiac surgery that had transfused RBCs filtered before storage to the non-WBC-reduced arm, non-WBC-reduced ABT was associated with a 72% increase in posttransfusion mortality (summary odds ratio [OR], 1.72; 95% confidence interval [CI], 1.05–2.81; p < 0.05; see Figure 62.3). The TRIM effect in these studies could be associated with factors prevalent in the setting of patients undergoing cardiac surgery. For example, bioactive lipids that accumulate during the storage of non-WBC-reduced RBCs and/or allogeneic mononuclear cells may represent a second inflammatory insult that may compound the diffuse inflammatory response associated with the cardiopulmonary bypass circuit, which may predispose recipients to MOF and mortality.

**Insights into the mechanism(s) of the TRIM effect(s) in cardiac surgery and trauma**

During cardiac surgery, exposure to the extracorporeal bypass circuit, hypothermia, and reperfusion injury can generate a systemic inflammatory response syndrome (SIRS) that is counteracted by a compensatory anti-inflammatory response syndrome (CARS). Any intervention by biologic response modifiers (e.g., soluble mediators) contained in stored non-WBC-reduced RBCs (e.g., as described under the “Mediators Originating in WBC Granules” section) during an already-existing inflammatory cascade could thus produce an imbalance in the SIRS–CARS equilibrium toward SIRS. An overwhelming SIRS causes a dormant state of cell metabolism referred to as multiple-organ dysfunction syndrome (MODS), which can ultimately lead to MOF and death. However, an association between non-WBC-reduced ABT and MOF has not been reported by any RCT, and the mechanism by which non-WBC-reduced ABT (but not WBC-reduced ABT) is associated with increased mortality in cardiac surgery remains unknown. In the completed cardiac-surgery RCTs (Figure 62.3), non-WBC-reduced ABT was not associated with any particular cause of death, yet the aggregate mortality was higher in the non-WBC-reduced arm than in the WBC-reduced arm.

Bilgin et al. investigated the pro- and anti-inflammatory cytokine profiles in patients participating in their cardiac-surgery RCT that compared recipients ofbuffy-coat-reduced versus WBC-reduced autologous RBCs. Patients who developed postoperative infection had higher interleukin-6 (IL6) concentrations, and patients who developed MODS had higher IL12 concentrations, in the subgroup of subjects who received more than three non-WBC-reduced RBC units. These findings supported the authors’ thesis that non-WBC-reduced ABT amplifies an inflammatory response that is a “second hit” superimposed upon the ongoing SIRS induced by cardiac surgery. Such a “second-hit” inflammatory response may subsequently lead to a more profound CARS, which amounts to transfusion-induced immunosuppression predisposing to enhanced susceptibility to postoperative infection.

Bilgin et al. also presented a combined observational analysis of the data from the two Dutch RCTs conducted in cardiac surgery. After adjusting for confounding factors in the combined...
data set, it was the plasma (rather than the RBC) transfusions that were associated with higher mortality in patients undergoing open-heart surgery. Non-WBC-reduced RBC transfusion was also significantly associated with postoperative infection. The authors concluded that, although it is difficult to separate the effects of the concomitantly administered allogeneic blood components (non-WBC-reduced or WBC-reduced RBCs, platelets, and plasma), future ABT studies in cardiac surgery should consider the possible adverse effects of all these various transfused blood components.

All platelets transfused in the two Dutch RCTs in cardiac surgery had been WBC-reduced. Bilgin et al. underscored the independent effect of WBC-reduced platelet transfusions on mortality observed in their observational combined analysis. Platelets expressing CD4L upon activation (in the extracorporeal circuit as well as during storage of the platelet components) are presumed to represent a vital link between coagulation and inflammation. As such, they may enhance microthrombi and venous thromboembolism, in particular under changing rheological conditions such as those that occur in cardiac surgery. Both thrombi and infection play a pivotal role in the development of MODS and mortality.

The link between plasma and platelet transfusion and mortality found in the latest cardiac-surgery analysis of Bilgin et al. is an observational finding that needs to be examined in future studies. Although this finding is indicative of a TRIM effect in cardiac surgery independent of allogeneic WBCs and not abrogated by WBC reduction, it should be borne in mind that an observational analysis cannot establish an effect of platelet and/or plasma transfusion that is independently associated with postoperative complications; instead, any demonstrated effect of platelet and/or plasma transfusion may be simply a marker of the effect of the concomitantly administered non-WBC-reduced RBC transfusions. The established finding from cardiac-surgery RCTs (Figure 62.3) thus remains that non-WBC-reduced (compared with WBC-reduced) RBC transfusion increases short-term postoperative mortality. Therefore, cardiac surgery is an established indication for WBC reduction.

More recently, there have been challenges to the two-hit SIRS–CARS model postulated by Bilgin et al. to account for the mechanism of the TRIM effect(s) in cardiac surgery. Jackman et al. studied immunomodulation in transfused trauma patients and delineated distinct roles of trauma and ABT in inducing immune modulation post injury. They demonstrated broad shifts in the expression of soluble immune mediators following traumatic injury and ABT, including early anti-inflammatory responses in contrast with the later anti-inflammatory (hence, immunomodulatory) responses envisioned by the SIRS–CARS model.

Xiao et al. found that, of the 20,720 genes investigated, expression of 16,820 (>80%) was significantly altered in blood WBCs after blunt trauma, appropriately naming this response a “genomic storm.” Early responses involved an increase in the expression of genes regulating innate immunity, microbial recognition, and inflammation, but also in anti-inflammatory mediators such as those involved with the IL10 signaling pathway. Jackman et al. found a similar scope of responses at the protein level, with the levels of 31 of 41 measured serum proteins significantly altered following trauma. Taken together, these studies demonstrate that the immune response to traumatic injury is incredibly broad, with significant changes observed in a majority of the genes and proteins assessed. Moreover, in contrast with the SIRS–CARS model of inflammation after trauma, these findings indicate immunosuppression occurs immediately after injury, coincident with some proinflammatory elements.

In fact, most of the largest anti-inflammatory responses, at both the RNA and protein levels, were seen at the earliest time points examined. Such an early response was mirrored in the mouse model of Jackman et al., with similar cytokine profiles seen at four hours after traumatic blood loss.

Although the major immunological event for transfused trauma patients appears to be the injury, ABT does influence this response. Xiao et al. found approximately 400 genes whose expression was dependent on the volume of blood transfused, most of which were downregulated in response to ABT. Jackman et al. found that, following adjustment for other clinical variables such as injury type and severity, seven of 41 proteins measured were significantly different between patients receiving modest ABT versus no ABT. An additional three proteins measured were significantly higher among those receiving ≥5 RBCs compared with the modest ABT group.

Studies “before-and-after” WBC reduction

In the late 1990s, Canada and many western European countries implemented universal WBC reduction of cellular blood components by means of prestorage filtration. It became possible to compare the risk of infection or mortality in recipients of non-WBC-reduced RBCs before implementation of WBC reduction with the risk of infection or mortality in recipients of WBC-reduced...
RBCs after implementation of WBC reduction. Such observational studies cannot establish causal relationships. Five studies have reported data on the risk of infection and/or short-term mortality.111

A large Canadian study included 9525 patients undergoing cardiac surgery, 1731 patients undergoing orthopedic surgery, and 3530 patients admitted to the intensive care unit (ICU). A statistically significant (p = 0.04) decrease in short-term mortality (from 7.0% to 6.2%) after WBC reduction was found but without a concomitant reduction in the risk of postoperative infection. The data-derived hypothesis offered was that the observed decrease in the number of deaths was not mediated through suppression of the recipient’s immune function, but through a pro-inflammatory microvascular effect of transfused WBCs that affects several organ systems. This hypothesis was buttressed by the findings of a companion before-and-after study in premature infants.113 In that setting, the implementation of universal WBC reduction coincided with a reduction in several secondary morbidity outcomes from several organ systems (i.e., bronchopulmonary dysplasia, retinopathy of prematurity, and necrotizing enterocolitis)—an observation consistent with a diffuse pro-inflammatory microvascular effect of allogeneic WBCs.

However, when all before-and-after studies were considered together in a meta-analysis,111 and the findings of the unadjusted analyses from five studies were integrated, there was an unadjusted association of WBC reduction with a decreased risk of postoperative infection. This association did not persist when findings from the multivariable analyses of the three observational studies that had adjusted for the effects of confounding factors were integrated. There was neither an unadjusted nor an adjusted association of WBC reduction with decreased short-term mortality.111

**Effect of the length of RBC storage**

If bioactive lipids or other soluble mediators accumulating in a time-dependent manner during storage were associated with adverse outcomes, an association between prolonged storage of transfused non-WBC-reduced allogeneic RBCs and increased risk of occurrence of these adverse outcomes should be seen (because the longer the non-WBC-reduced allogeneic RBCs are stored, the higher the level of such soluble mediators that they will contain). Also, the RBC storage lesion (Chapter 9) occurs in both the non-WBC-reduced and the WBC-reduced units, generating considerable numbers of nonfunctioning RBCs removed from the recipient’s circulation within 24 hours of the transfusion. This places a considerable burden on the reticuloendothelial system of a multi-transfused recipient that could interfere with the host’s response to bacteria and other challenges. After RCTs from 1993 to 2004 of non-WBC-reduced versus WBC-reduced ABT reported no adverse TRIM effect(s) vis-à-vis cancer recurrence and postoperative infection (with the exception of cardiac surgery), investigation focused on the possible deleterious effects of the transfusion of “old” (versus “fresh”) RBCs.

The Age of Blood Evaluation (ABLE) RCT114 enrolled approximately 2500 intensive-care unit (ICU) patients in Canada to detect a 5% absolute risk reduction (from 25% to 20%) in the mortality of ICU patients associated with transfusion of fresh (versus old) RBCs. Patients were randomly assigned to receive RBCs stored for ≤7 days versus RBCs issued per standard blood-bank policy (which issues for transfusion the longest-stored compatible RBC unit available in the inventory). The Red Cell Duration Study (RECESS)115 intended to compare 1434 US cardiac-surgery patients randomly assigned to receive RBCs stored for ≤10 versus ≥21 days. The study evaluated change in the multiple-organ dysfunction score (MODS; from preoperative to highest composite MODS, adjusted for baseline MODS). The Age of Red Blood Cell in Premature Infants (ARIPI) RCT116 recruited 450 premature neonates in Canada and allocated them to receive RBCs stored for ≤7 days versus RBCs issued per standard blood-bank policy. ICU patients, cardiac-surgery patients, and premature neonates were considered to be most vulnerable to any deleterious effect. These three RCT failed to show a difference between the study cohorts who received fresh versus old RBC transfusions. However, also in Canada, Heddle et al.117 are simultaneously conducting a “pragmatic” RCT that admits all hospitalized transfused patients to detect an adverse effect of old RBCs in an unselected population not restricted to ICU patients, cardiac-surgery patients, or neonates. This last RCT117 is intended to enroll 25,000 patients to detect a 15% reduction in mortality in association with transfusion of fresh (compared with old) RBCs.

In 1999, Vamvakas and Carven reported specifically on the TRIM effect of RBC storage. In an observational study of patients undergoing cardiac surgery, 54 of 416 (13.0%) patients developed postoperative pneumonia. Among 269 patients given RBCs, the risk of pneumonia increased by 1% per day in the mean storage time of the transfused RBCs (p < 0.005). In an analysis of all patients, the risk of pneumonia increased by 5% per unit of non-WBC-reduced allogeneic RBCs and/or platelets received, although this difference did not attain significance (p = 0.06).118 No association of the length of RBC storage was detected with such surrogate and nonspecific indicators of postoperative morbidity as the postoperative length of hospitalization, the postoperative length of stay in the ICU, and the length of endotracheal intubation after the day of operation.119

Numerous observational studies were published subsequently and have been critically reviewed.120–123 These observational studies attributed to the transfusion of old (rather than fresh) RBCs such common (and nonspecific) adverse outcomes as a greater risk of mortality and organ failure (often analyzed by means of such surrogate variables as length of hospitalization and length of stay in the ICU) in addition to in-hospital infection. Furthermore, various other mechanisms were proposed to account for the postulated deleterious effect(s) ascribed to old RBCs. These include procoagulant124 and/or immune125 effects of old (versus fresh) RBCs secondary to the development of microparticles in old blood, increased iron load from hemolyzed stored RBCs,126 and/or depletion of nitric oxide127 or S-nitrosylated hemoglobin128 in stored RBCs causing reduced ability of the transfused RBCs to induce vasodilatation (thereby resulting in inadequate blood flow and impaired oxygen delivery).129

Further mechanisms were proposed by Cata et al.130 These authors attributed the TRIM effect(s) primarily to the effect(s) of RBC storage, proposed mechanisms to account for a continuing deleterious effect of stored RBCs after the implementation of WBC reduction, and focused especially on the tumor-promoting effect(s) of stored RBCs (whether WBC-reduced or not).130 They proposed that transfusion of allogeneic blood components (RBCs, but also platelets and fresh frozen plasma) is associated with a pro-inflammatory burden of bioactive substances in the recipient. The extent of this pro-inflammatory load in the recipient seems to be proportional to the length of storage of the transfused RBCs. Many of these bioactive substances have the potential to directly and indirectly affect the innate immune function (natural-killer [NK] cell activity) in the recipient—a key protective mechanism for local tumor...
control and also directed against metastatic spread in the surgical patient. The question therefore is whether the acute inflammatory burden produced by the perioperative ABT in the immunosuppressive environment of anesthesia and surgical trauma creates a pro-tumor environment for the establishment of distant metastases.\[130\]

Cytokine concentrations are significant in stored WBC-reduced RBC units.\[131\] Exposure of WBC-reduced stored RBC supernatant to whole blood triggers release of IL6, IL10, and tumor necrosis factor-\(\alpha\) (TNF\(\alpha\)).\[132\] This reduces lipopolysaccharide-induced release of TNF\(\alpha\),\[133\] and induces regulatory T-cell (Treg) activation.\[134\] Treg cells (comprising some 1–2% of circulating CD4-positive T-helper cells that coexpress a very high density of the IL2 receptor-\(\alpha\) [CD25\textsuperscript{hi}]) inhibit IL2 production and suppress the functions of T-helper type 1 (Th1) responses by CD4 and CD8 T cells.\[135\]–\[137\] The activation of Treg cells is antigen nonspecific, because they can be activated by lipopolysaccharide and can become immunosuppressive through the Toll-like receptor-4 pathway.\[138\]

Furthermore, a recent animal study\[139\] found that RBCs (rather than WBCs) are implicated in the cancer-promoting effects of both autologous and allogeneic blood transfusions, with prolonged storage of the transfused RBCs enhancing tumor progression. However, clinical studies have hitherto suggested that neither prolonged RBC storage\[140\]–\[142\] nor administration of non-WBC-reduced (versus WBC-reduced) RBCs\[143\]–\[144\] is associated with any increased risk of cancer recurrence.

All this evidence\[131\]–\[139\] suggests that administration of stored blood components may be more deleterious than the administration of fresh blood components from a proinflammatory/immunomodulatory perspective and that universal WBC reduction may not abrogate the proinflammatory/immunomodulatory effects of ABT.\[130\] If the association of old (versus fresh) RBCs with the common adverse outcomes investigated in the recently completed\[114\]–\[116\] or still ongoing\[117\] RCTs of the effect of the length of RBC storage were shown to be causal, the allowed storage period of RBCs would have to be promptly reduced, and increased reliance would have to be made on patient blood management (PBM) approaches because the patient’s own freshly shed blood is the freshest blood possible.\[145\] Prompt policy intervention would be required because the effect of old (versus fresh) RBCs on common adverse outcomes reported from some observational studies\[146\]–\[150\] far exceeds the risks from the reduction in inventory and the need to recruit additional first-time donors, as well as the cost of producing more RBC units to make up for the expected increase in outdated units after the allowed RBC storage period is reduced. Thus, the question of whether old blood is less safe than fresh blood was appropriately the most critical issue facing transfusion medicine in 2011.\[151\] However, the results of three of the four RCTs undertaken in the United States or Canada have been consistently negative.

The negative findings of the RCTs of RBC storage\[114\]–\[116\] had been correctly predicted by all four critical reviews of the available observational clinical studies.\[120\]–\[123\] These reviews questioned the soundness of the clinical evidence adduced to justify the undertaking of RCTs. More specifically, in observational studies, any effect of RBC storage appeared to be a surrogate factor for the number of RBC units transfused, which in turn was a surrogate factor for illness severity predisposing to a need for transfusion.\[122\]–\[125\] Thus, in the ABLE RCT,\[114\] 37.0% of patients receiving fresh RBCs had died at 90 days, as compared with 35.3% of patients receiving old RBCs. The hazard ratio for death in recipients of fresh (compared with old) RBCs was 1.1 (\(p = 0.38\)). There were no significant differences between the randomization arms in any of the secondary outcomes. In the RECESS,\[115\] the main change in MODS was an increase of 8.5 and 8.7 points, respectively, in recipients of fresh versus old RBCs (\(p = 0.44\)). Seven-day mortality was 2.8% versus 2.0%, respectively (\(p = 0.43\)); 28-day mortality was 4.4% versus 5.3% (\(p = 0.57\)). Adverse outcomes did not differ significantly between the randomization arms. In the ARIPI RCT,\[116\] the relative risk of the primary outcome (a composite of major neonatal morbidities) was exactly 1.00. Thus, a slight, non-significant trend favored old RBCs as the better component to transfuse in the ABLE RCT,\[114\] and fresh RBCs in the RECESS,\[115\] with no difference whatsoever observed in the ARIPI RCT\[116\]; exactly what is expected when no clinical difference exists between old and fresh RBCs.

**TRIM effects mediated by soluble molecules circulating in allogeneic plasma**

**Soluble HLA molecules**

Soluble HLA proteins and immunoreactive HLA peptides are possible mediators of the TRIM effect(s). Nonpolymorphic peptides derived from HLA class I molecules might induce antigen-nonspecific immunosuppression, whereas polymorphic HLA class I peptides have antigen-specific immunomodulatory effects.\[152\] It also seems possible that allogeneic plasma containing soluble HLA antigens may enter the recipient’s thymic circulation, producing clonal deletion of the recipient’s T cells that are directed against the allogeneic donor antigens.\[156\]

**Factor VIII concentrates**

In vitro studies have indicated that low-molecular-weight components found in factor VIII concentrates may inhibit the proliferative responses of peripheral blood mononuclear cells to phytohemagglutinin.\[157\] In these studies, high-purity factor VIII concentrates reduced the induction of T-cell-activation molecules such as the CD25, the transferrin receptor (CD71), CD38, the CD11a–CD18 ratio, and HLA-DR antigen expression.\[158\] This inhibitory action of factor VIII concentrates was at least partly due to their contamination by transforming growth factor (TGF)-\(\beta\).\[159\]

**Autoantibodies**

Some people spontaneously produce large amounts of neutralizing autoantibodies to a number of growth factors (e.g., granulocyte-macrophage colony-stimulating factor) or cytokines (e.g., IL1, IL6, and interferon-\(\alpha\)).\[160\] The autoantibodies in question are detectable in immunoglobulin preparations and in plasma for transfusion. Therefore, if such donors donate blood, large amounts of neutralizing autoantibodies to growth factors or cytokines may be transferred to recipients by transfusion. In the plasma of transfusion recipients who had received plasma from donors with high titers of high-affinity neutralizing autoantibodies to IL6 (0.1% of normal donors), Hansen et al.\[160\] demonstrated a 500-fold or greater increase in the concentration of complexed IL6/autoantibody to IL6, as compared with free IL6 detectable in the patients’ plasma prior to the transfusion.

When these autoantibodies reach a certain level, they may render a donor (or the recipient of plasma from such a donor) cytokine-deficient, but overt clinical sequelae of such cytokine
deficiency have not been reported. Hansen et al.\textsuperscript{139} christened this phenomenon \textit{transfusion-related inhibition of cytokines} (TRICK). Depending on the cytokine or growth factor involved, TRICK could conceivably increase the transfusion recipient’s susceptibility to infection or delay hematopoietic recovery after stem cell transplantation.

**Evidence from RCTs**

One RCT\textsuperscript{76} design permitted examination of the hypothesis that soluble plasma molecules circulating in allogeneic plasma may mediate TRIM effects. Wallis et al.\textsuperscript{76} randomized 597 patients undergoing cardiac surgery to receive plasma-reduced, buffy-coat-reduced, or WBC-reduced allogeneic RBCs. Plasma-reduced RBCs are equivalent in WBC content to the buffy-coat-rich RBCs used in North America. The highest risk of postoperative infection was observed in the plasma-reduced arm, in which the incidence of postoperative infection was 17.1%, as compared with 10.8% in the buffy-coat-reduced arm and 11.3% in the WBC-reduced arm ($p = 0.20$). Although the difference between the three arms was not significant, plasma removal did not appear to confer a benefit with regard to the prevention of TRIM (i.e., allogeneic plasma did not mediate TRIM).

**TRIM effects mediated by WBC-derived soluble mediators**

**Mediators originating in WBC granules**

Biologic response modifiers accumulating in blood components during storage have been implicated in the pathogenesis of TRIM.\textsuperscript{160} These mediators are contained in intracellular WBC granules, and are released in a time-dependent manner as the WBCs deteriorate.\textsuperscript{161} Nielsen et al.\textsuperscript{161} reported that the concentration of histamine, eosinophil cationic protein, eosinophil protein X, myeloperoxidase, and plasminogen activator inhibitor-1 increase 3- to 25-fold in the supernatant fluid of RBC components between days 0 and 35 of storage. Histamine, eosinophil cationic protein, and eosinophil protein X have been shown to inhibit neutrophil function, thereby contributing to the development of immunosuppression and tissue damage.\textsuperscript{162,163}

**Soluble HLA molecules and Fas ligand**

Soluble HLA molecules are present in the serum or plasma of healthy individuals. The liver is the main source of soluble HLA molecules found in the circulation. High levels of these molecules have been found in the serum or plasma of transplanted patients and patients with a variety of conditions, including inflammatory, autoimmune, and infectious diseases. Soluble HLA molecules are also found in the supernatant fluid of stored RBCs and platelets, in direct proportion to the length of storage and the number of cells present. The biological significance of these molecules has not been fully established, although it has been reported that they may be involved in the downregulation of the immune response and/or induction of tolerance.

Ghio et al.\textsuperscript{164} and Puppo et al.\textsuperscript{165} found soluble Fas-ligand (sFasL) and soluble HLA class I molecules in the supernatant plasma of RBC and random-donor platelet units. The sFasL content of either 30-day stored RBCs or 5-day stored platelets was approximately 20 ng/ml. The infusion of sFasL in transfused blood components may bind the Fas molecule expressed on the NK and cytotoxic T-cells of the recipient, thus preventing the binding of the Fas molecule on these immune cells to the Fas-ligand on virus-infected cells. Therefore, the infusion of sFasL in transfused blood components may impair the function of NK and cytotoxic T-cells in the recipient, thus preventing apoptosis of virus-infected cells.\textsuperscript{166,167} Ghio et al.\textsuperscript{164} and Puppo et al.\textsuperscript{165} demonstrated the functional capacity of sFasL molecules in stored blood components by culturing Jurkat cells in the presence of plasma supernatant from stored RBCs. Jurkat cells express Fas, and are thus susceptible to the effects of sFasL present in transfused blood components. In this in vitro experiment, sFasL from the plasma supernatant of stored RBCs triggered apoptosis of the Jurkat cells, which was measured by flow cytometry.

These authors\textsuperscript{164,165} also documented the accumulation of soluble HLA class I molecules in stored RBCs and platelets, although the concentrations achieved were only 4 ng/ml in 30-day stored RBCs and five-day stored platelets. Furthermore, stored supernatant plasma was shown to exercise an immunosuppressive effect in functional experiments, inhibiting the cytotoxic activity of lymphocytes known to be cytotoxic for cells infected with Epstein–Barr virus (EBV). This was not a nonspecific effect, as the cytotoxic activity of lymphocytes was restored after the stored supernatant plasma was depleted of soluble HLA class I molecules. However, only supernatant plasma from stored non-WBC-reduced (as opposed to WBC-reduced) cellular blood components inhibited the cytotoxic activity of lymphocytes directed against EBV-infected cells. Similarly, prestorage WBC reduction prevented the accumulation of sFasL in stored RBCs.

**Apoptotic WBCs**

Innerhofer et al.\textsuperscript{168,169} reported that impaired proliferative T-cell responses, decreased CD3+ counts, and a state of inappropriate immune activation, along with a diminished cytolytic response, occur even after transfusion of WBC-reduced RBCs containing a median residual WBC count of 0.03 × 10\(^6\) WBCs/unit (i.e., a count far below the qualifying 5 × 10\(^6\) limit). This suggests that not only transfused, intact, immunologically competent WBCs but also transfused, apoptotic, or necrotic WBCs could be important in provoking TRIM responses. Finally, activation of complement components\textsuperscript{170} and formation of anaphylatoxins\textsuperscript{171} have also been reported during storage, but their significance in the context of TRIM is uncertain.

**Evidence from animal models**

If soluble biologic response modifiers and remnants of apoptotic or necrotic WBCs accumulating in blood components during storage were shown to be responsible for some of the adverse TRIM effects of ABT, WBC reduction procedures intended to prevent such TRIM effects should be performed before storage, prior to WBC deterioration and prior to the release from WBC membranes or granules of soluble biologic response modifiers (Figure 62.1). The available WBC reduction filters do not retain soluble biologic response modifiers and are also ineffective in removing WBC fragments. Therefore, both biologic response modifiers and the remnants of apoptotic or necrotic WBCs can be expected to persist in a blood component filtered after storage, and the importance of the timing of WBC reduction as regards the TRIM effects has been demonstrated in experimental animals.\textsuperscript{172} Bordin et al.\textsuperscript{172} showed that ABT promotes tumor growth of established animal tumors and that the tumor-growth-promoting effect of ABT can be ameliorated by prestorage (but not by poststorage) WBC reduction. These authors\textsuperscript{172} used outbred
New Zealand White (NZW) rabbits with established tumors as blood recipients, and outbred California Black rabbits as allogeneic blood donors. “Syngeneic” donor blood was collected from NZW rabbits who were littermates, or siblings, of the transfusion recipients. Non-WBC-reduced allogeneic, poststorage WBC-reduced allogeneic, prestorage WBC-reduced allogeneic, or syngeneic RBCs were transfused on days +4 and +9 after the infusion of syngeneic epithelial tumor cells. All rabbits were killed 28 days after the infusion of the tumor cells, and the number of pulmonary tumor nodules was counted. Rabbits that received non-WBC-reduced allogeneic, poststorage WBC-reduced allogeneic, prestorage WBC-reduced allogeneic, or syngeneic RBCs before WBCs could release mediators into the supernatant was only marginally significant (p < 0.0001), but the difference between non-WBC-reduced allogeneic and poststorage WBC-reduced allogeneic transfusion was only marginally significant (p = 0.06).

**Evidence from RCTs**

With respect to infection, a recent theory\(^{173,174}\) attributes the purported susceptibility of transfused patients to infection to a sustained inhibition of neutrophil chemotaxis caused by TGFβ. TGFβ renders neutrophils insensitive to chemotactic stimulation. Inhibition of chemotaxis is caused by both exogenous TGFβ, contained in the supernatant of transfused blood components,\(^{173}\) and endogenous TGFβ produced by the recipient’s neutrophils in response to sFlsL and soluble HLA molecules found in the transfused supernatant.\(^{174}\)

However, the results of meta-analyses of medically homogeneous subsets of RCTs that reported on postoperative infection contradicted the theory that attributes the TRIM effect to WBC-derived soluble mediators that accumulate during storage.\(^{57,58}\)

Among nine RCTs transfusing RBCs WBC-reduced before storage to the WBC-reduced arm,\(^{62,68,70,73,76–80}\) no TRIM effect was detected (summary OR, 1.06; 95% CI, 0.91–1.24; p > 0.05).\(^{57}\) If the TRIM effect were mediated by WBC-derived soluble mediators, prestorage filtration should have abrogated an increased infection risk associated with non-WBC-reduced ABT, because it would have removed the allogeneic WBCs from the components given to the WBC-reduced arm of the studies before WBCs could release mediators into the supernatant fluid. Accordingly, a deleterious TRIM effect associated with non-WBC-reduced ABT would have been expected in this analysis, but the meta-analysis detected no such effect (Figure 62.4).

In contrast, among four RCTs\(^{59,64,68,69}\) that transfused RBCs filtered after storage to the WBC-reduced arm, there was a more than twofold increase in the risk of infection in association with non-WBC-reduced ABT (summary OR, 2.25; 95% CI, 1.12–4.25; p < 0.05; Figure 62.5). If the TRIM effect were mediated by WBC-derived soluble mediators, poststorage filtration should not have abrogated an increased infection risk associated with non-WBC-reduced ABT, because it would not have removed such mediators from the supernatant fluid of the stored RBCs given to the WBC-reduced arm of the studies. Thus, the large TRIM effect detected in this analysis (Figure 62.5) may be due to the inclusion of three early RCTs\(^{59,64,69}\) that had reported an unusually large TRIM effect.\(^{58}\)

These RCTs administered blood components that are no longer used in Western Europe or North America (allogeneic whole blood,\(^{59}\) poststorage-filtered allogeneic whole blood,\(^{69}\) or poststorage-filtered allogeneic RBCs\(^{64,69}\)).

**Figure 62.4** Risk of postoperative infection after transfusion of prestorage-filtered WBC-reduced RBCs. Randomized controlled trials (RCTs) of ABT and postoperative infection administering prestorage-filtered allogeneic RBCs to the WBC-reduced arm.\(^{62,68,70,73,76–80}\) The figure shows the odds ratio (OR) of postoperative infection in recipients of non-WBC-reduced versus WBC-reduced allogeneic RBCs, as calculated from an intention-to-treat analysis of each study; and the summary OR across the depicted RCTs, as calculated from a meta-analysis.\(^{57}\) A deleterious ABT effect (and thus a benefit from WBC reduction) is demonstrated by an OR >1, provided that the effect is statistically significant (p < 0.05; i.e., provided that the associated 95% CI does not include the null value of 1).

**TRIM effects mediated by allogeneic mononuclear cells**

The only established TRIM effect (i.e., the beneficial effect of pretransplant ABT on renal allograft survival) appears to require viable WBCs. Patients awaiting renal transplantation derived less immunologic benefit from pretransplant RBC transfusions that are WBC-reduced, washed, or frozen-thawed. Mincheff et al.\(^{177}\) implicated the dendritic APCs of the allogeneic donor in the induction of a state of anergy in the recipient, proposing that during refrigeration APCs lose their ability to deliver co-stimulation. These investigators hypothesized that, following ABT, the recipient’s T cells are stimulated by allogeneic donor APCs in the absence of co-stimulation, and this interaction induces a state of anergy in the recipient’s T cells.

**Evidence from animal models**

Animal data suggest that the TRIM effects are most likely mediated by transfused allogeneic mononuclear cells.\(^{176}\) Kao\(^{177}\) induced immune suppression in mice receiving donor WBCs free of plasma and platelets. A recent theory\(^{178}\) proposes that donor dendritic cells expressing both alloantigen and the OX-2 (CD200) co-stimulatory molecule are required for the production of the TRIM effect.

CD200 is a transmembrane protein of the immunoglobulin superfamily that is expressed on various cell types, including a subpopulation of dendritic cells and some T and B cells.\(^{179}\) Its receptor (CD200R or OX-2R) appears only on myeloid dendritic cells and some T cells. The interaction between CD200 and its receptor provokes a tolerance signal that leads to suppression of classical T-cell-mediated responses and the generation of
γδ-suppressor T cells. Thus, CD200-deficient mice have reduced ability to downregulate activation of APCs. The absence of downregulatory signals in such mice results in exaggerated inflammatory responses and increased susceptibility to autoimmune encephalitis and collagen-induced arthritis. The interaction between CD200 and its receptor suppresses macrophage function, prolongs allograft survival, and prevents allogeneic fetal loss in a mouse model of cytokine-triggered abortions.180

Clark et al178 demonstrated ABT-induced tumor growth in a murine model that employed BALB/c mice as allogeneic donors and C57Bl/6 mice as blood recipients. The recipient mice received a tail vein injection of syngeneic FSL10 fibrosarcoma cells, followed by transfusion of 50–200 μL of allogeneic blood. Pulmonary tumor nodules were counted three weeks after the FSL10 cell infusion. There was a dose–response relationship between the volume of transfused allogeneic blood and the number of pulmonary tumor nodules, along with proliferation of TGFβ-positive suppressor T cells in the spleen.

The tumor-growth-promoting effect of ABT was mediated by donor myeloid dendritic cells that expressed both CD11c and CD200 on their surface, because it could be blocked by monoclonal antibodies to either CD11c or CD200. (The effect could not be blocked by antibodies to CD200R, or by antibodies to other molecules participating in these interactions, an observation that implicated the subset of donor myeloid dendritic cells expressing both CD11c and CD200 in the pathogenesis of TRIM.) The interaction between the donor CD200 and its receptor on the recipient’s T cells induced proliferation of γδ-suppressor T cells that released cytokines, especially TGFβ. Physiological concentrations of TGFβ stimulated proliferation of FSL10 fibrosarcoma cells in vitro. As TGFβ can also suppress host defenses against infectious agents,181 it could be the basis of the TRIM effect with regard to both postoperative infections and tumor growth, at least as regards sarcomas.

Bordin and Blajchman182 reviewed the findings of animal models of ABT and cancer recurrence and reported that 17 published models had found stimulation of tumor growth by ABT, as compared with three models that had reported inhibition of tumor growth and four models that had found no effect. Data from both inbred and outbred animal models have indicated that ABT accelerates tumor growth and enhances formation of metastatic nodules.172,183–186 Allogeneically transfused mice inoculated intramuscularly with either syngeneic malignant melanoma (B16) or mastocytoma (P815) cells developed larger tumors than did syngeneically transfused mice.183 Similar results were obtained when syngeneic B16 tumor cells were infused intravenously and the numbers of pulmonary nodules enumerated.183,184 Experiments performed to investigate the effect of the tumor–cell dose showed that the ABT effect was only evident when small numbers (1.25–2.5 × 10^6) of tumor cells were inoculated into the host animal. The effect was not evident when large numbers of tumor cells were inoculated, suggesting that the tumor burden had a strong bearing on whether the ABT effect became manifest.

Studies in both inbred (mice) and outbred (rabbits) animals have shown that ABT has a tumor-growth-promoting effect when administered prior to the infusion of syngeneic tumor cells.172,185 In the murine model, male C57Bl/6 (MHC type H-2b) mice were blood recipients, Balb/c mice (MHC type H-2d) were allogeneic donors, and the tumor cells were syngeneic (H-2b) methylcholanthrene-induced fibrosarcoma cells.185 To better replicate the situation seen clinically, the enhancement of tumor growth by ABT has been investigated in animals (mice and rabbits) that received such syngeneic and allogeneic transfusions subsequent to the inoculation of the tumor cells, and the data indicated that ABT enhanced tumor growth also in animals with established tumors.172,185 In the murine model, male C57Bl/6 mice (MHC type H-2b) were blood recipients, Balb/c mice (MHC type H-2d) were allogeneic donors, and the tumor cells were syngeneic (H-2b) methylcholanthrene-induced fibrosarcoma cells.185 Is was also shown that animals with either non-established or established tumors receiving non-WBC-reduced ABT developed significantly larger numbers of pulmonary nodules than did animals given WBC-reduced ABT.172,185

Finally, the tumor-growth-promoting effect of ABT can be adoptively transferred to naive animals by spleen cells harvested from allogeneically transfused animals.185 In these experiments, the number of pulmonary nodules observed in animals that had received spleen cells from allogeneically transfused animals was significantly higher than that observed in animals that had received spleen cells from animals transfused with syngeneic blood. Importantly, the ABT effect could not be adoptively transferred to naive animals that received spleen cells derived from animals transfused with prestorage-WBC-reduced allogeneic blood.

The clonal deletion seen in recipients of ABT refers to the removal of lymphocytes that promote the clearance of transfused alloantigens. Interactions between Fas and FasL are involved in the clonal deletion of T cells and the downregulation of cytotoxic T-cell activity. In a murine model, Hashimoto et al.187 investigated the

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Figure 62.5 Risk of postoperative infection after transfusion of poststorage-filtered WBC-reduced RBCs or whole blood. Randomized controlled trials (RCTs) of ABT and postoperative infection administering poststorage-filtered allogeneic RBCs or whole blood to the WBC-reduced arm. The figure shows the odds ratio (OR) of postoperative infection in recipients of non-WBC-reduced versus WBC-reduced allogeneic RBCs or whole blood, as calculated from an intention-to-treat analysis of each study; and the summary OR across the depicted RCTs, as calculated from a meta-analysis. A deleterious ABT effect (and thus a benefit from WBC reduction) is demonstrated by an OR >1, provided that the effect is statistically significant (p < 0.05; i.e., provided that the associated 95% CI does not include the null value of 1).
possibility of splenic-lymphocyte deletion secondary to ABT-related augmentation of apoptosis. These investigators demonstrated that non-WBC-reduced ABT upregulated the expression of Fas and FasL on CD4+ as well as CD8+ splenic T cells and could thereby promote their apoptosis. The ABT-related immune alterations could be partially prevented by WBC reduction of the transfused blood, as CD8+ splenic cells from mice receiving non-WBC-reduced ABT showed higher expression of Fas and FasL than cells from mice receiving WBC-reduced ABT.

The data regarding the TRIM effect in animal models of infection are contradictory. Moreover, a variety of experimental conditions such as the anesthesia, presence of shock or trauma, type of surgery, blood volume, as well as timing and transfusion frequency have all been reported to have an impact on the results.\textsuperscript{188–192} In a series of studies in experimental animals, Waymack et al. have demonstrated that allogeneically transfused animals had immune impairment and a poorer response to a septic challenge than did syngeneically transfused animals.\textsuperscript{188–189} In a burn model, these investigators observed that rats given ABT had higher mortality than did rats given syngeneic blood or saline.\textsuperscript{192} In a rat bacterial-peritonitis model, a significant adverse effect on survival was associated with ABT.\textsuperscript{189} In another study, ABT was associated with marked immune impairment to a bacterial challenge immediately after the transfusion.\textsuperscript{193}

Moreover, ABT and, in particular, transfused allogeneic WBCs adversely affected host resistance to a gut-derived infection with \textit{Escherichia coli} in a murine model.\textsuperscript{194} In addition, in a cecal ligation and puncture murine model, ABT greatly increased susceptibility to infection. These studies also indicated that spleen cells of allogeneically transfused mice produced increased quantities of the Th2 cytokines (IL4, IL10, and lesser amounts of IL2), probably leading to increased antibody production and a decreased cell-mediated response.\textsuperscript{195} In contrast, in murine experiments using a bacterial-peritonitis model that compared syngeneic with ABT, the latter was shown not to influence overall survival of animals challenged with \textit{E. coli}.\textsuperscript{196} Although a clear negative effect of shock was detected, no adverse effect of transfusions, either syngeneic or allogeneic, was observed in a rat model.\textsuperscript{197}

Microchimerism

HLA compatibility between donor and recipient may result in the persistence of allogeneic donor WBCs, including dendritic APCs, in the recipient. Such long-term engraftment and survival of small numbers of donor cells (microchimerism) have been proposed as a possible mechanism of TRIM.\textsuperscript{198} Microchimerism could cause the downregulation of the recipient’s immune response, resulting in tolerance to donor alloantigens and allograft survival. Microchimerism results in the release of IL4, IL10, and TGF\textbeta{} from Th2 lymphocytes.\textsuperscript{199} These cytokines have been shown to inhibit the production of Th1 cells and to deactivate cytotoxic T cells, thereby suppressing allograft rejection. Along similar lines, ABT was shown to cause a shift in peripheral T-cell cytokine secretion toward that of the Th2 phenotype, and to downregulate Th1 cytokine secretion. Impairment of Th-1 cytokine secretion results in impairment of various functions of cellular immunity (including antigen processing, macrophage activation, the T-cell cytotoxic function, and the neutrophil and monocyte cytotoxic activity) that are supported by Th1 cytokines, such as IL2, IL12, and interferon-\gamma.\textsuperscript{200}

In 1995, Lee et al.\textsuperscript{201} employed quantitative allele-specific polymerase chain reaction methods to demonstrate a 1000-fold expansion of allogeneic donor WBCs in the recipient’s circulation 3–5 days following transfusion in otherwise healthy adults undergoing elective orthopedic surgery. The allogeneic WBCs were cleared from the circulation within two weeks. The finding was verified in a canine transfusion model, and—as expected—irradiation of blood products abrogated the allogeneic donor WBC expansion phase. However, in 1999, the same group documented high-level and long-lasting WBC microchimerism among selected victims of traumatic injury who had received a large number of very fresh units of blood during resuscitation.\textsuperscript{202} In some of these trauma patients, up to 3–4% of circulating WBCs were of donor origin as long as two years following transfusion. Analysis of lymphocyte subsets using immunomagnetic bead enrichment showed that both lymphoid and myeloid lineages were represented.

Long-term transfusion-associated microchimerism appears to be a common, albeit only recently recognized, complication of ABT\textsuperscript{203} that has hitherto been demonstrated only in trauma patients.\textsuperscript{202} Injury produces an immunosuppressive and inflammatory milieu in which very fresh blood components, containing WBCs capable of replication, are often transfused in large quantities. Transfusion-associated microchimerism is present in approximately half of transfused, severely injured patients at hospital discharge.\textsuperscript{203} In approximately 10% of the patients, the chimerism associated with a single blood donor may increase in magnitude over months to years, representing up to 2–5% of circulating WBCs.\textsuperscript{202,203} Nonetheless, in other patient populations, such as those infected with HIV, ABT-induced microchimerism is transient.\textsuperscript{204}

Microchimerism is detected also following administration of WBC-reduced allogeneic RBCs.\textsuperscript{205} Utter et al.\textsuperscript{206} examined a subgroup of the trauma patients enrolled in the RCT of Nathens \textit{et al.}\textsuperscript{80} that had randomized patients to receive non-WBC-reduced or WBC-reduced allogeneic RBCs filtered before storage. Nine of 32 (28%) patients in the non-WBC-reduced group developed microchimerism, as compared with 13 of 35 (37%) patients in the WBC-reduced group ($p = 0.43$).\textsuperscript{206} Several months after the transfusion, subjects with transfusion-associated microchimerism were no more likely than subjects without transfusion-associated microchimerism to have at least one symptom suggestive of chronic graft-versus-host disease (64% versus 76%, respectively), indicating that transfusion-associated microchimerism is prevalent in this patient population but unlikely to be associated with symptoms.\textsuperscript{206} Fresland \textit{et al.}\textsuperscript{207} also reported that microchimerism after ABT could be induced by transfusion of RBCs WBC-reduced by prestorage filtration.

The only significant predictor of transfusion-associated microchimerism to date is the length of storage of transfused RBCs, with “fresh” (compared with “old”) RBCs associated with a higher risk of microchimerism.\textsuperscript{208} Reed \textit{et al.} reported a significantly ($p < 0.05$) different RBC storage time between non-transfusion-associated microchimerism recipients (21 ± 8.3 days) versus transfusion-associated microchimerism recipients (16.1 ± 6.2 days).\textsuperscript{203} Similarly, the minimal length of RBC storage was a median of 13 days in non-transfusion-associated microchimerism recipients compared to a median of five days in transfusion-associated microchimerism recipients ($p < 0.005$).\textsuperscript{203} Nonetheless, transfusion-associated microchimerism has also been observed with older RBCs (stored for 22 days).\textsuperscript{207} If transfusion-associated microchimerism were related to TRIM effects in trauma, and were also associated with transfusion of “fresher” RBCs, it is important to appreciate that it would be the transfusion of fresh (as opposed to old) RBCs that
would be logically associated with adverse TRIM effects in trauma. Nonetheless, no adverse clinical effects have hitherto been observed in the small number of completed studies of transfusion-associated microchimerism, indicating any TRIM effects in trauma are not related to transfusion-associated microchimerism.

**Evidence from RCTs**

One RCT was specifically designed to test a possible TRIM effect of allogeneic mononuclear cells. The Viral Activation Transfusion Study (VATS) transfused unmodified allogeneic RBCs stored for less than 4 weeks (and thus containing relatively undamaged allogeneic mononuclear cells) to the non-WBC-reduced arm. The two study arms were controlled for comparable duration of storage of the transfused RBCs. There was no difference between the study arms in the HIV or CMV viral load or the length of survival. Median survival was 13.0 months in recipients of prestorage-filtered allogeneic RBCs, compared with 20.5 months in recipients of non-buffy-coat-reduced allogeneic RBCs (p = 0.12). Thus, VATS results have impugned the theory attributing the TRIM effect to allogeneic mononuclear cells. No other RCT transfusing fresh components to the non-WBC-reduced arm has been reported, and the effect of fresh components has not been studied in the context of more “traditional” TRIM effects (i.e., cancer recurrence or postoperative infection).

Despite the convincing evidence provided by animal models for a relationship between transfusion of allogeneic mononuclear cells and tumor recurrence, no RCT of ABT and cancer recurrence has transfused fresh, non-WBC-reduced RBCs to the non-WBC-reduced arm to test for the effect of immunologically competent allogeneic mononuclear cells seen in animal models. Moreover, no RCT of ABT and cancer recurrence has enrolled patients with sarcomas—tumors whose growth is stimulated by TGF-β—nor patients with tumors for which the immune response plays a major role. (These include skin tumors, such as melanomas, keratoacanthomas, and squamous and basal-cell carcinomas; and certain virus-induced tumors, notably Kaposi’s sarcoma and certain lymphomas.)

Instead, the three available RCTs of ABT and cancer recurrence enrolled patients with colorectal cancer—a tumor that is not sufficiently antigenic to render an impairment of the host’s immunity capable of facilitating tumor growth, and whose cells have not been shown to be stimulated by TGF-β. The existence of a specific immune response to colorectal cancer cells has not been established. Although it is possible to generate cytotoxic T cells in vitro that recognize antigens expressed by colorectal cancer cells, the relevance of these cytotoxic cells in tumor growth may be limited because of a loss of the expression of HLA molecules and adhesion molecules on the colorectal cancer cells. Furthermore, if the ABT cancer-promoting effect were mediated by allogeneic WBCs, the dose of ABT used in each of the three available RCTs could have been insufficient for causing the adverse effects because all three RCTs had administered buffy-coat-reduced RBCs to their non-WBC-reduced arm.

Thus, these 3 RCTs permit very limited inference with regard to the biologic significance of the TRIM mediators discussed in this chapter. No adverse TRIM effect of ABT on cancer recurrence is detected across the three studies (Figure 62.6). The summary OR of cancer recurrence in recipients of non-WBC-reduced allogeneic compared with the autologous or WBC-reduced allogeneic RBCs is 1.04 (95% confidence interval [CI], 0.81–1.35; p > 0.05). A fourth RCT was recently reported in gastrointestinal cancer.

### Summary and conclusions

TRIM encompasses the laboratory immune aberrations that occur after ABT (Table 62.1) and their established or purported clinical effects. TRIM is a real biologic phenomenon resulting in at least one established beneficial clinical effect in humans (the enhanced survival of renal allografts), but deleterious clinical TRIM effects have not yet been confirmed. Initially, TRIM encompassed effects attributable to ABT by immunomodulatory mechanisms (e.g., cancer recurrence, postoperative infection, or virus activation); more recently, TRIM has also included effects attributable to ABT by pro-inflammatory mechanisms (e.g., multiple-organ failure or mortality).

The mechanism(s) of the TRIM effect(s) remain(s) elusive, and it is possible that a large number of biologic mechanisms underlie these effect(s). The infusion of foreign antigen in either soluble or cell-associated form has been shown to induce immune suppression, anergy, as well as clonal deletion in studies in experimental animals. However, most studies evaluating proposed mechanisms have been done in rodents, and their findings may not be applicable to the human immune system. Support for the theory that TRIM
is due to the allogeneic WBCs has come mainly from data from animal models. These have shown that animals receiving allogeneic buffy-coat leukocytes develop significantly more pulmonary tumor nodules than do animals given either plasma or prestorage-WBC-reduced whole blood.172 It is possible that prestorage WBC reduction may prevent the accumulation of soluble mediators that are actively synthesized and released by WBCs during RBC storage, and that such WBC-derived soluble mediators are involved in the immunomodulation observed following ABT. However, storage lesions of RBCs and platelets occur even when the units have been WBC-reduced prior to storage (WBC removal only slightly improves these storage lesions—see Chapters 9 and 24).

The totality of the evidence from RCTs does not demonstrate the kind of deleterious TRIM effect that would justify universal WBC reduction specifically for prevention of this effect (i.e., a TRIM effect manifest across all clinical settings and transfused RBC products), although universal WBC reduction may be justified on the basis of other WBC-related adverse effects.176 Non-WBC-reduced ABT is associated with an increased risk of short-term (up to three months post transfusion) mortality from all causes specifically in cardiac surgery. Even in this setting, the reasons for the excess deaths attributed to non-WBC-reduced ABT remain elusive. The initial hypothesis suggested that non-WBC-reduced ABT may predispose to MOF, which, in turn, may predispose to mortality.68 However, hitherto, no cardiac-surgery RCT has demonstrated an association between non-WBC-reduced ABT and MOF.

The TRIM effect seen in cardiac surgery deserves further study to pinpoint the cause(s) of the excess deaths, but RCTs comparing recipients of non-WBC-reduced versus WBC-reduced RBCs in cardiac surgery are not likely to be performed. We believe that WBC reduction of all cellular blood components transfused in cardiac surgery is appropriate based on the accumulated evidence on the adverse TRIM effect(s) in this specific clinical setting. Where selective WBC reduction is practiced, WBC reduction of cellular components transfused in cardiac surgery should be added to the other established indications for selective WBC reduction.

The evidence for the existence of some TRIM effects may not be available because the requisite studies have not been conducted. An effect of the transfusion of allogeneic mononuclear cells would be expected to increase tumor recurrence, based on the convincing findings from experimental animals. However, no available RCT has transfused fresh non-WBC-reduced RBCs to the non-WBC-reduced arm to specifically study the effect of allogeneic mononuclear cells. Moreover, no available RCT has enrolled patients with a tumor whose growth would be expected to be stimulated by ABT. A possible adverse TRIM effect of allogeneic mononuclear cells has similarly not been adequately investigated in the areas of postoperative infection and mortality.

Indeed, in many cases, the preclinical studies were conducted and the hypotheses about mechanisms formulated after clinical studies (including RCTs) had already presented data-derived hypotheses to account for unexpected ABT effects. Because it has not been possible to conduct further RCTs after the hypotheses about TRIM mediators (Figure 62.1) were crystallized, we may never know whether some adverse TRIM effects exist (or not) in humans, because we have been unable to test for them in RCTs. Moreover, it is possible that the available RCTs have targeted outcomes that did not capture the true nature of the ABT effect. If this effect were “pro-inflammatory” rather than “immunomodulatory,” it would have been expected to result not in clinical impairment of the recipient’s immunity, but in multiple-organ dysfunction. MOF and related outcomes were not studied in most completed RCTs.

Following a great interest in TRIM in the 1990s and the early years of the twenty-first century (when the adverse TRIM effects were debated as the primary reason for implementing universal [as opposed to selective] WBC reduction in North America—if not in both North America and Western Europe), remarkably few clinical studies on the adverse TRIM effects (and even the mechanisms of these effects) appeared in the last seven years. This is partly due to the fact that each country’s policy decisions vis-à-vis implementing universal WBC reduction had already been made in the early years of the 21st century,216,217 it is also partly due to the funding of research into the deleterious effects of stored RBCs in lieu of research into the bona fide TRIM effects.

It remains unclear whether deleterious clinical TRIM effects of ABT truly exist, and—in the event that they do—whether they are mediated, directly or indirectly, by allogeneic WBCs. The question of whether universal WBC reduction should be discontinued and selective WBC reduction reintroduced was posed after no new research was reported to justify the policy decisions made in favor of WBC reduction in the early years of the twenty-first century (when there was an implicit expectation that adverse TRIM effects would later be established).211 Some US transfusion medicine experts have argued against such a change in clinical practice on the grounds that patients and clinicians would not agree with the change.218 Whereas ardent believers in TRIM have continued to argue in favor of universal WBC reduction,219 Bilgin, van de Watering, and Brand220 presented a concise and sobering review of the evidence from RCTs that separated experimental evidence from speculation, conviction, faith, and belief.

Based on the stark absence of any adverse ABT effect in RCTs outside the setting of cardiac surgery, these authors concluded that reversal to the use of buffy-coat-reduced RBCs (with restriction of WBC reduction to its established selective indications) is a “safe option.”220 The data presented in this chapter support the scientific merits of this position,216 which is further supported by the latest results from the three-year follow-up221 of 2016 patients randomized to receive “liberal” versus “restrictive” ABT in the RCT of Functional Outcomes in Cardiovascular Patients Undergoing Surgical Hip Fracture Repair (FOCUS) trial.222 Over three years of follow-up, 841 (42%) patients died. Long-term mortality did not differ between the “liberal” and “restrictive” ABT arms of the RCT (hazard ratio, 1.09; 95% CI, 0.95–1.25), and causes of death did not differ either, providing no support for the hypothesis that a liberal transfusion strategy (and thus ABT) has an adverse effect on long-term mortality or affects the causes of death.221

Key references

A full reference list for this chapter is available at: http://www.wiley.com/go/simon/transfusion

Section V: Part II: Other hazards


