Transfusion-Related Immunomodulation

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INTRODUCTION

Blood transfusions represent to date one of the most common clinical approaches to treat patients with a myriad of etiologies that lead to anemia, thrombocytopenia, or deficient coagulation requiring factor supplementation. With a growing aging population, the demands on transfusion medicine facilities and donation centers are likely to continue to increase in the coming years. However, use of blood either whole or its components in the treatment of patients is not without risks. One such entity that has been the reason of heightened concern over the last 40 years is known as transfusion-related immunomodulation (TRIM). Importantly, the question we continue to ask is if this should still be a matter of concern today when blood components are safer than they have ever been. Considering that in the United States there is a red blood cell (RBC) transfusion every 2 seconds that translates into approximately 16 million units transfused in the country on an annual basis,[1] immunomodulatory adverse events if common could represent a major public health issue that would need to be addressed by the medical community.

RBC transfusions in particular have been the focus of years of research to understand the potential effects that they may have once transfused in the recipient. Specifically, transfusion of RBC units results in immune exposure to cell-bound and cell-free antigens or metabolites that lead to changes in a recipient's immune response.[2] These effects can also potentially occur when transfused with either pooled or single-donor derived platelet units. Therefore, in this chapter, data will be presented to guide the reader to take into consideration those changes that blood component preparation have undergone since the initial studies were published decades ago. With this in mind, studies will be presented in the context of how components were prepared at the time for transfusion so that there is a better understanding of the effects that these components may have had and to facilitate comparison of findings from older studies to more recent ones.

HISTORICAL PERSPECTIVE

As mentioned earlier, it has been known for quite some time that RBC units can contain immunomodulatory mediators that lead to changes in the way the immune system of a recipient responds posttransfusion. However, this potentially complex response to blood transfusions needs to be properly defined to grasp its true effects especially in those critically ill who may be particularly susceptible to potentially deleterious effects of transfusions in either the adult or the pediatric settings.[3,4] In its simplest form, TRIM can be seen as those potentially proinflammatory or immunosuppressive effects that can occur because of a transfusion due to mediators that are preformed and present in the unit or that are produced by the recipient as a response to the transfusion.

First, we should step back in time and look at the early reports that first described these potential effects of blood. It is agreed upon in the literature that it was the report in the 1970s by Opelz and colleagues of lower rejection rates of patients who underwent renal transplantation and received allogeneic transfusions, which the authors argued was secondary to lower concentrations of antihuman leukocyte antigen (HLA) antibodies post-RBC transfusion, that provided the foundation for TRIM.[5] This observation resulted in deliberate transfusions of transplant patients regardless of need to have better posttransplantation outcomes; this is something that would be more difficult to do today.[6] Nevertheless, this important finding would define the next 40 years in how blood transfusions were seen and utilized in clinical settings.

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Early data from animal models appeared to indicate that transfusion of blood made more likely metastasis of solid organ malignancies.[7,8] However, this association since then has been disputed by others who have not seen such an association; as a result, this still remains a matter of debate.[4] Nevertheless, it can be said that any degree of immunosuppression by blood transfusions as that defined as TRIM would have detrimental effects in other clinical settings such as patients dealing with malignancies, either in remission or not, and those patients fighting infections.[9–12] Undoubtedly, early studies indicating that the post-transfusion risk of infection was greater also inferred that this correlated with the number of units transfused, especially, when compared to infectious rates of patients who did not receive transfusions.[13,14] Therefore, this risk had to be taken into consideration when the decision to transfuse was made. However, specific variables such as patients' complex and significant health impairments, and lack of risk-stratification when determining the effects of transfusion in relation to a patient's condition may have had immediate bearing on the effects seen in these early studies.

NEWER PERSPECTIVES

Despite increased awareness, this has not stopped the controversy of how to best define TRIM and attempts have been made to limit its definition to those secondary to length of storage of RBC units (Fig. 6.1).[15] In this redefinition, the focus shifts almost exclusively to the changes that RBC units undergo while in storage and the deleterious effects of using these older RBC units.[15] This distinction, however, allows to differentiate TRIM from those processes that lead to alloimmunization to RBC antigens and/or iron overload secondary to iron present in RBC units; similarly, it also sets forth that this and any other definition should be limited to the effects of blood components over the function of the recipient's immune system. In support of this definition, it has been reported that the length of storage causes physiological and metabolic changes that can result in deleterious effects as shown in an in vivo model of transfusion comparing stored versus fresh RBCs. In this model, mortality and tumor progression were not mediated either by the leukocytes still present in the transfused blood or by the RBC supernatant but instead by the aged RBC themselves.[16] Additionally, depending upon the timing of the patient's response,

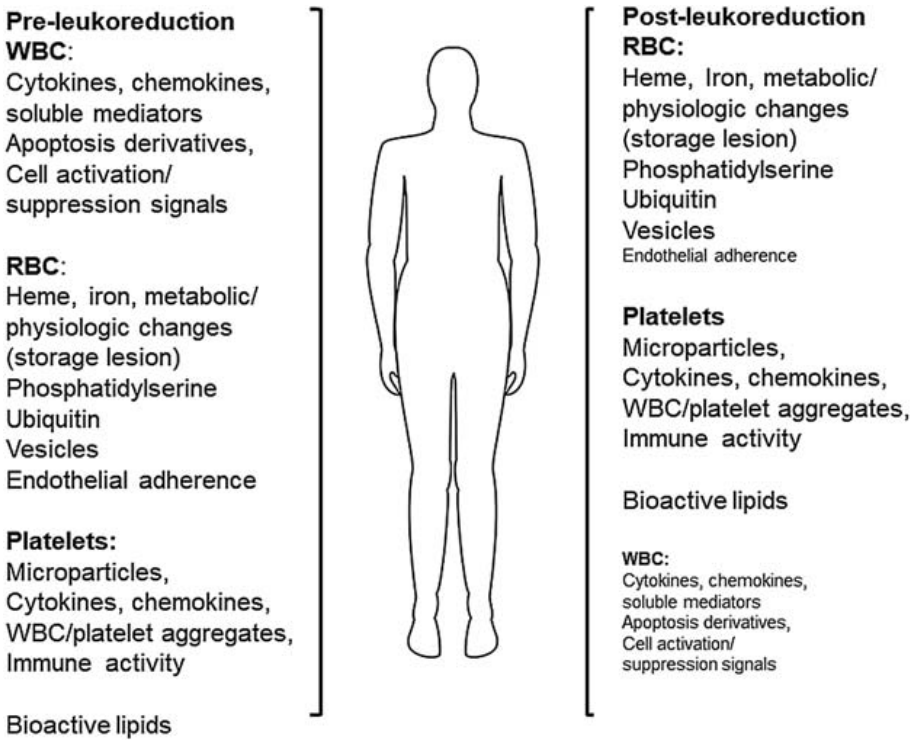

FIG. 6.1 Diagram of role of each blood cell or soluble mediator potentially involved in TRIM before and after prestorage leukoreduction. Smaller font postleukoreduction signifies decreased role of WBC-mediated factors leading to TRIM.

RBC transfusion effects could be seen as part of a continuum that are either immediate or delayed, and in some cases elicit memory responses that result in reactions to other RBC units at a later time point.[15]

Existing clinical evidence has also described at least temporal association between RBC transfusions and higher incidence of organ dysfunction, nosocomial infections in addition to possible cancer recurrence.[17] Reports have also indicated that the remaining plasma supernatant of stored RBCs may increase the metastatic (immunosuppressive) effects of transfusion,[18,19] which in animal models could be worse when cells/plasma are derived from female mice.[20] However, opinions of this potential association have changed over the years as it became clearer that remaining white blood cell (WBC) in RBC units, or factors released by them, mediated at least some of the negative effects secondary to RBC transfusions (Fig. 6.1). Over time, this drove home the argument for wide use of leukoreduced RBC units.[21–23] Nevertheless, despite greater awareness immunomodulatory effects are still seen, though less frequently, in the postleukoreduction era.

BIOLOGICAL MECHANISMS

The cumulative weight of the data indicates that these mechanisms are likely multifactorial. RBC transfusions appear to activate a recipients' WBC resulting in decreased neutrophil chemotaxis, phagocyte activation, exacerbation of a patient's limited immune response, and cytokine dysregulation.[4,17,24,25] Of these, neutrophil chemotaxis has been shown to be mediated by RBC supernatants containing transforming growth factor (TGF)-β, and these inhibitory effects can be replicated either by using plasma from RBC recipients (containing endogenous TGF-β among other mediators) or by using exogenous TGF-β (Fig. 6.2).[26]

An interesting hypothesis that is supported by increasing literature is that blood represents a "second immunological hit" that brings about those physiological changes characteristic of TRIM.[25] This is similar to what has been suggested in severe transfusion reactions such as transfusion-related acute lung injury,[27] which is the focus of discussion elsewhere in this book. This implies that there must be a primary insult to the immune system that predisposes the transfusion recipient to TRIM. Therefore, even though the focus of research has been to some extent limited to the composition/content of a unit, the condition of the recipient is equally relevant when one analyzes data reporting these adverse events to transfusion.[28] Unfortunately, this may be the one unintended bias introduced by studies reporting TRIM that will prove difficult to eliminate. This would explain why a meta-analysis of available randomized trials and of the literature found the data inconsistent to establish if RBC storage influences TRIM; however, data from this analysis did point out that when these adverse events do occur they may be limited to specific patient subpopulations such as those in cardiac settings and/or trauma.[29] This is one area that will be discussed later in this chapter.

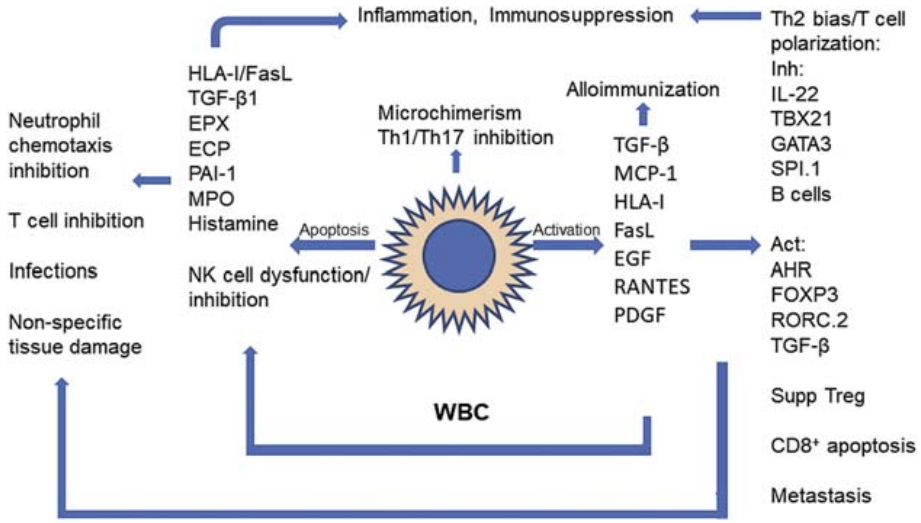

FIG. 6.2 Representation of white blood cell–driven mediators and mechanisms leading to TRIM. To the right, representation of processes dependent upon WBC activation and to the left those processes driven by cell apoptosis. Arrows represent the flow of mediators and the potential role in a particular immunomodulatory process. *Inh*, inhibition; *Act*, Activation; *Supp*, Suppressor.

Regarding immunosuppressive effects, a number of cellular and tissue targets can be affected in a recipient because of transfusions, which explains the profound systemic effects that have been reported. For example, it has been shown that RBC transfusions inhibit natural killer (NK)-cell activity and that this is mediated by decreases in soluble HLA-I, soluble Fas ligand (L), and changes in production of TGF-β.[30] Similarly, in a time-dependent manner, nonleukoreduced RBC units showed a bias toward greater number of T helper (h)2 cells versus Th1 (Fig. 6.2). This occurs in the setting of decreases in transcription factors TBX21, GATA3, SPI.1, and cytokine IL-22 with concomitant increases in AHR, FOXP3, RORC.2, and TGF-β.[31] Of note, regardless of leukoreduction, RBC units appear to induce proliferation of suppressive regulatory T cells suggesting that, at least regarding this effect, leukoreduction may have limited effectiveness.[32]

Over the years, studies looking at the effect of leukocytes in transfused units focused on patients undergoing cardiac surgery who needed transfusions. These studies indicated that patients who received nonleukoreduced units were more likely to have complications during their hospitalization and increased mortality,[33] and in surgical patients have a higher propensity for infections.[13,34] Authors of these studies, therefore, favored leukoreduction of RBC units to avoid such complications. Larger studies and meta-analyses have also pointed out that leukoreduction results in significant reduction in complications and mortality in adults.[23,35] However, a similar benefit of leukoreduction in mortality was not observed in transfused low-birth-weight premature patients, but use of leukoreduced units did reduce the incidence of bacteremia, bronchopulmonary dysplasia, retinopathy, intravascular hemorrhage and necrotizing enterocolitis.[36] Taken together, these reports indicated that the effects over the immune system in critical patient settings appeared dependent upon the leukocyte load in units that were not leukoreduced.[37]

One important element that earlier studies alluded to is that the patient population receiving blood is an important variable in TRIM occurrence as likely recipient factors play a major role in the responses to blood components regardless of leukoreduction. If an analogy could be made, this is similar to what has been seen in the infectious diseases literature in which microorganisms normally innocuous to the general population as a whole become pathogenic in immunocompromised patients. As a result, as patients who receive blood tend to be more severely ill at the time of transfusions this inevitably adds factors that may be exacerbated and "prime" the recipient to react negatively to a blood transfusion. This possibility could provide a physiological explanation to the early discrepant results observed in randomized clinical trials looking at the effect of transfusion in blood recipients.[2]

Leukocytes in the blood unit are also involved in processes leading to alloimmunization (Fig. 6.2). Evidence indicating that leukocytes in transfused blood also play a role in RBC alloimmunization have come from mouse studies indicating that apoptotic WBCs in transfused blood release factors that regulate RBC alloimmunization such as TGF-β, and by among other mechanisms causing polarization of naïve $CD4^+CD25^+$ T cells.[38] This is not at all unexpected, because it has been known for some time that RBC units have increases in inflammatory cytokines in a time-dependent manner during storage.[39,40] Leukoreduction does result in marked decreases in these soluble mediators.[39] However, data of the immunosuppressive effects of increasing TGF-β levels secondary to apoptotic WBCs in the unit does not exclude endogenous production of this cytokine by recipients' macrophages as shown post-transfusion of RBCs expressing phosphatidylserine that results in immunosuppression.[41]

T-cell activation and deactivation are likely to mediate either the reported proinflammatory or the immunosuppressive effects of RBCs. This has been suggested by data indicating that transfusion of both fresh and RBCs stored for longer periods leads to suppression of cytokine production by isolated T cells in vitro.[42] Nevertheless, of the two, older RBCs result in greater decreases in cytokine production including interleukin (IL)-10, IL-17a, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and granulocyte macrophage colony-stimulating factor compared to fresh RBCs.[42] However, RBCs may not need to be stored for long time periods to exert an immune effect. RBCs stored for as little as 2−3 weeks can inhibit both $CD4^+$ and $CD8^+$ T cells stimulated with anti-CD3/CD28, inhibit B cells stimulated with lipopolysaccharide, and both of these effects could be reversed by transfusing fresh RBCs.[43] Similarly, comparisons of transfusions of fresh versus older RBCs in a mouse model have indicated that the latter results in increased IL-6, keratinocyte-derived chemokine/CXCL1 and monocyte chemoattractant protein-1.[24]

OLD VERSUS FRESH UNITS

Based on what was described in the prior paragraph, older RBCs seem to result in higher incidence of TRIM compared to fresh cells. Yet, the data indicating this

remain controversial. As mentioned earlier, responses to RBCs may not be totally dependent upon storage times as a trend toward less mortality has been described using stored RBCs.[29] Later on, authors of this earlier study reanalyzed observational data and came to a markedly different conclusion that though RBC units stored greater than 30 days did not correlate with greater incidence of nosocomial infections, mortality did appear to correlate with longer RBC storage.[44] These conflicting results exemplify the difficulties in determining the true effect of RBC storage.

This debate between fresh versus not fresh units leading to different patient outcomes has been reason for concern in the mind of clinicians for decades. Indeed, there is data describing that decreased deformability of erythrocyte membranes may occur because of storage duration but more importantly that this change can continue even after cells are transfused.[45] This later point is an important one as it would imply that older units would not provide full therapeutic benefit due to their storage age. However, results from randomized clinical trials may shed more light into this issue and their results will be discussed next.

Randomized clinical trials provide the best evidence regarding the role that the age of the unit and changes related to the storage lesion can cause in patients. A recent large multinational trial, the Age of Blood Evaluation (ABLE), which included European and Canadian medical centers found that use of fresh RBC units (7 day or less) provided no survival advantage and did not lead to significant economic benefit compared to standard issue RBC units.[46] Even though the focus of this large trial of over 1200 patients in each investigational arm was not pediatric patients, among those included in the study were teenagers, and results were similar to those described for the whole study population. Similarly, the Age of Red Blood Cells in Premature Infants trial indicated that the age of RBC units did not have a noticeable effect on outcomes among neonatal patients.[47] Similarly, the Red-Cell Storage Duration Study that looked at outcomes in cardiac patients undergoing surgery and required transfusions did not show that outcomes of patients receiving units >21 days were worse than those patients receiving fresh units.[48] Finally, the findings reported by the ABLE trial were also similar in critically ill adults requiring transfusion.[49] One major randomized clinical trial in pediatric patients that at the time of writing this chapter is still underway is The Age of Blood in Children in Pediatric Intensive Care Unit and its results hopefully in the near future will provide further insight into the potential differences between older versus fresher units in this patient population.[50] As a result, based on the information conveyed by these large trials, the age of the unit may not necessarily be a determinant of poor outcomes in diverse patient populations.

Despite results obtained by these trials, differing opinions of the role of older versus fresh RBC units in pediatric settings have not ended with the previously mentioned data. Contrary to what has been described by others, in the pediatric setting transfusion of older (>21 days) RBC units has been reported to lead to persistence of systemic inflammation and innate immunity suppression (measured by ex vivo lipopolysaccharide stimulation leading to TNF-α production) when compared to patients receiving fresher units or fewer number of units.[51] In this same study, IL-6 production was decreased by use of fresher RBC units. The differences in this study's observations could be due in part to being observational in nature, have a relatively small patient cohort while the ones listed earlier were well-controlled randomized clinical trials. This inevitably adds variables to each study that are likely to add confounders leading to markedly different conclusions. Along these lines, additional reports have found an association between the age of RBC units and high incidence of complications including mortality in both adult and pediatric cardiac patients in intensive care units.[52–54] Similarly, there is literature indicating that older RBC units lead to innate immunity (cytokines) suppression.[55–58] This immune suppression would result in higher infectious rates and mortality as shown in trauma patients receiving transfusions that could be made worse by transfusing a greater number of units.[59,60] Something that will require closer investigation in future studies is what role higher levels of IL-10 play in TRIM responses. This is because in patients who have had repeated reactions to blood components, they are found to have increased concentrations of IL-10 upon transfusion suggesting that in this cohort this may represent one of the mechanisms leading to immunomodulation (Fig. 6.3).[61] Likely, this contentious topic will continue and future studies will need to be carried out to provide further insight and resolve the role that metabolic and cellular changes that occur while in storage play in TRIM.

WBCS IN UNITS AND LEUKOREDUCTION

As eluded earlier, WBC in the unit can produce mediators that lead to the unforseen reactions that qualify as TRIM. It should be of interest to the reader that in some instances these negative effects have been described as lasting long after transfusion exposure. For example, it was reported that 2 weeks posttransfusion, plasma samples from blood recipients had high levels of TGF-β1

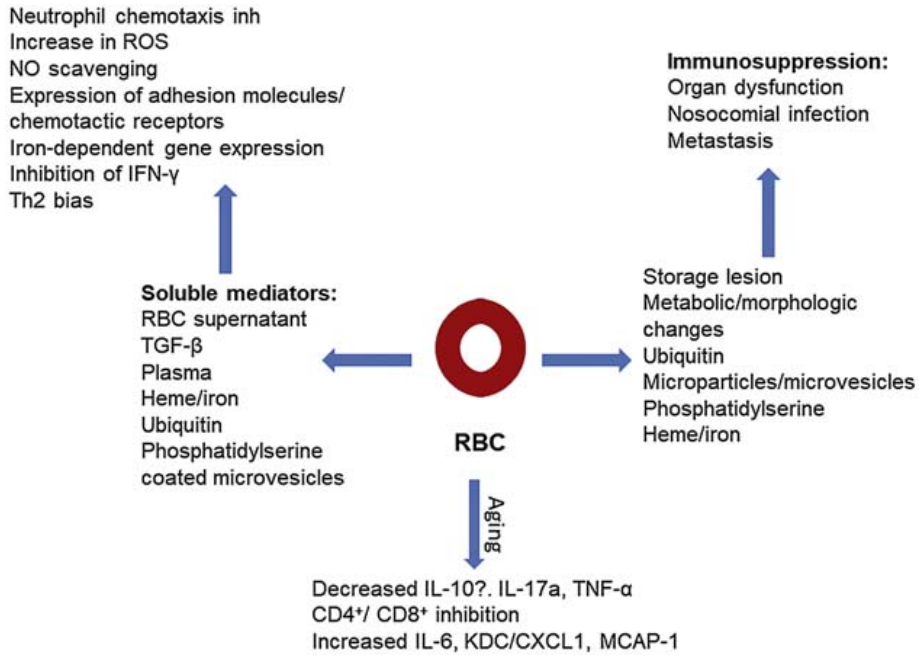

FIG. 6.3 Representation of red blood cell—mediated mechanisms described in TRIM. Aging represents data referring to age of unit. To the right, potential effects secondary to storage lesion are indicated. To the left, mechanisms possibly mediated by soluble mediators are indicated.

synthesized by recipients' neutrophils that were the result of direct interaction with soluble FasL and HLA-I molecules present in nonleukoreduced transfused units (Fig. 6.2).[62] This in turn inhibits neutrophil chemotaxis leading to increased susceptibility to infections. Similarly, blood units derived from placentas have high TGF-β1 produced by apoptotic or necrotic WBC that increases over time, and this may be associated with erythrocyte lysis in a time-dependent manner.[63] This mechanism also appears to mediate the response to intravenous immunoglobulin through induction of TGF-β1 that is also mediated by soluble FasL and HLA-I,[64] suggesting that these mediators are involved in multiple immunomodulatory responses. Interestingly, soluble HLA-I free chains increase in both RBC and platelet units during storage, are produced by remaining WBC in the units in a time-dependent manner, and at some threshold cause apoptosis of $CD8^+$ T cells suggesting that this could be an additional immunosuppressive effect that has been described in both allogeneic and autologous blood transfusions.[65]

RBC units also increase perioperative inflammatory responses as described in patients receiving transfusions during cardiopulmonary bypass surgery,[66] and these effects can be lessened if leukoreduced RBC units are utilized.[67] These findings represented an additional reason to push for leukoreduction of units that has resulted in an overall decrease in the number of reports of potential immunomodulatory effects of blood, including associated mortality, organ dysfunction, and infectious rates.[22,23,68] Likewise, leukoreduction has translated into improved postsurgery survival of cardiac patients thanks to potentially lower proinflammatory rates.[69] However, despite benefits seen post leukoreduction, survival of patients transfused with leukoreduced units is still lower than patients who are not transfused, and regarding cancer recurrence rates it has shown not to have made a difference.[70] Nevertheless, reports from animal models indicate that use of leukoreduced blood results in fewer recipient splenic T cells undergoing apoptosis that is mediated by Fas/FasL,[71] and decreased rates of solid tumor metastasis.[72] As a result, reported results of positive benefits of restrictive transfusion strategies that minimize blood usage whenever possible can be understood, as this approach results in fewer nosocomial infections compared to patients liberally transfused.[73]

One point that needs to be reemphasized is that despite almost universal prestorage leukoreduction of units across the United States, up to 5×10^6 WBCs/unit still remain in the bag and these cells can still cause some of the reactions described thus far.[74–76] Despite initial data indicating the role that HLA-I played in some of the immunomodulatory processes mediated by WBCs in the unit, there is evidence that class II may

also mediate some of these effects. MHC class II molecules on antigen presenting cells (APCs) such as monocytes and dendritic cells in the donated unit are also capable of initiating a cascade of events that activate T cells and result in either immunosuppression or alloimmunization.[77,78] Immune responses can be further complicated by differences between a recipient's and donor's HLA antigens, and depending on the recipient's clinical condition lead to alloimmunization, or when costimulation is incomplete result in immune tolerance and T-cell anergy.[17] This latter effect provides for an explanation when microchimerism occurs after allogeneic blood transfusions, due to lack of an immune response, allowing allogeneic cells to survive in a recipient's circulation.[79] Survival of allogeneic cells could last for years posttransfusion due to engraftment of donor leukocytes as seen in multilineage microchimerism of trauma patients who received intense transfusion support (Fig. 6.2).[79,80] Mechanistically, the extent of this microchimerism is secondary to Th2 activation and TGF-β increases resulting in immunosuppression posttransfusion,[81,82] reduction in both Th1 and Th17 responses,[83] and even changes to patterns of gene expression needed for a healthy immune response.[84]

ROLE OF APOPTOSIS AND SOLUBLE MEDIATORS

Cell death and/or apoptosis can cause release of mediators that can be immunomodulatory in nature. One of these molecules released during cell death is phosphatidylserine that is expressed on RBC-derived microparticles and cellular debris produced postapoptosis.[85] Upon exposure to phosphatidylserine, synthesis of cytokines such as IL-10 and TGF-β increases, and inhibition of NFκB and p38 MAPK occur as early as 72 hours poststorage downregulating dendritic cells and activating T regulatory cells (Fig. 6.3).[86,87] These apoptotic effects, however, are not unique to RBC units, as they have also been reported to occur in platelet units as well.[88]

Apoptosis leads not only to cell fragmentation but also to the release of cell content including bioactive substances from cytoplasmic granules. Among such contents are histamine, eosinophil protein X (EPX), myeloperoxidase, eosinophil cationic protein (ECP), and plasminogen activator inhibitor 1 that are released by both leukocytes and platelets in a time-dependent manner during storage.[89] Importantly, these mediators are significantly decreased by prestorage leukoreduction.[90] Of these, histamine, EPX, and ECP have been shown to inhibit neutrophil chemotaxis and T lymphocyte proliferation in vitro,[91,92] and in some instances result in diffuse nonspecific tissue damage (Fig. 6.3).[93]

It has also been reported that supernatant of stored RBC units can suppress monocytes, and washing of units can remove soluble mediators responsible for this inhibition.[51,94] Since mediators that can cause TRIM symptomatology are found in solution and are released by remaining WBCs still present in RBC units, leukoreduction would not fully remove the potential risk represented by these mediators to cause unforseen adverse events in the recipient. Mediators such as soluble FasL and TGF-β that as mentioned earlier lead to TRIM,[26,38] and to a lesser extent soluble HLA-I molecules work through direct inhibition of a recipient's immune responses even when autologous RBC units are transfused.[64,95] These mediators can activate apoptotic signals, or result in dysfunctional neutrophil chemotaxis and NK cell activity.[1] This is one point that needs to be emphasized that use of autologous units does not exempt the recipient from reacting adversely to these soluble mediators which may have significant effects over a recipient's immune responses.

Additional soluble immune mediators in the form of cytokines can also be released by WBC present in the unit, although these are likely to be less of a factor in the era of leukoreduction. Data indicate that procarcinogenic cytokines such as monocyte chemotactic protein-1 (MCP-1), RANTES, epidermal growth factor, and platelet-derived growth factor increase over time during storage; however, all of these cytokines are markedly reduced but not eliminated through leukoreduction.[19] Therefore, it should be no surprise that in patients with impaired immunity such as young infants reports that MCP-1, IL-1β, IL-8, IL-17, TNF-α, IFN-γ, IFN-γ-induced protein 10, soluble intracellular adhesion molecule, and plasma macrophage inhibitory factor are increased in recipients posttransfusion. These mediators in some instances lead to TRIM-associated symptomatology in this patient population.[96,97]

HEME, IRON, AND RBC MEDIATORS

It is clear that prolonged storage results in RBCs, which as they complete their life cycle, release their contents into suspension and this may be increasingly more significant toward the end of the unit's shelf life. However, this is a constant process that starts at time of collection since donated RBCs when drawn from the recipient span in age from young to older RBCs as they enter the bag. As mentioned earlier, storage itself may cause changes to RBCs partly driving some of the deleterious changes associated with TRIM and increased mortality as shown in in vivo models.[98,99] These storage-length

changes define the storage lesion. These negative effects can be minimized by washing older units resulting in better animal survival, less lung injury, better cardiac and liver functional responses, and above all reduced levels of nontransferrin-bound iron and plasma labile iron.[100] Despite these findings, available data from randomized clinical trials does not support that storage significantly affects outcomes after transfusion that are likely dependent upon a recipient's clinical condition or a given patient subpopulation rather than to the age of units used.[101]

We therefore need to look at biochemical changes caused by storage to understand the potential mechanisms mediating the reported deleterious effects. Among changes that RBCs undergo in the bag are oxygen affinity, decreased pH, cell morphological changes, and increased membrane permeability[102]; fortunately, some of these can be reversed using RBC rejuvenation techniques that improve energy metabolism (Fig. 6.3).[103,104] However, these techniques cannot reverse the senescence of cells that continues as cells reach their expected lifespan. Nevertheless, despite these storage-dependent changes, reviews of the literature have failed to find evidence that fresh units are necessarily superior to older units[105,106]; and in some instances the opposite could be true.[107] Undoubtedly, the lack of uniformity in defining what an old unit is for a given population (adult vs. pediatric, trauma, surgery, numerous comorbidities among others) may prove a difficult obstacle to overcome and find consistency among reports showing that exposure to older units correlates with poorer outcomes.

However, two mediators are constantly released in a unit while in storage, and they are heme and iron. Presence of either one of these bioactive substances in solution at the time of transfusion could immediately influence the recipient's response to transfusion. Yet, the question that needs to be asked is if iron and/or heme can sufficiently affect the recipient's immune system to result in changes associated with TRIM. Furthermore, RBC units need not to be old to generate free hemoglobin and iron as phagocytes in the spleen among other organs can remove aging cells in fresher units via extravascular hemolysis.[108] Once free, iron deposition can occur in a variety of organs or by itself lead to inflammatory responses.[109] Both heme and iron can also cause tissue damage directly due to formation of reactive oxygen species (ROS), or indirectly through activation and increased migration of leukocytes via enhanced expression of adhesion molecules and chemotactic receptors.[110–112]

Iron released during storage can be found as transferrin-bound, nontransferrin-bound, or in plasma form.[1] Of these, nontransferrin-bound iron causes release of inflammatory cytokines in mice,[109] but such effects thus far have failed to be shown to occur in either adults,[113] or in pediatric patients.[114] In this latter patient group, however, transfusion itself and not iron specifically may be behind transfusion-associated neonatal enterocolitis,[97] and evidence points that MCP-1 mediates these immunomodulatory responses in neonates.[115] Different from iron, upon cell lysis free hemoglobin can be removed from circulation by the action of haptoglobin but this capability can be overwhelmed when the concentration of hemoglobin exceeds compensatory mechanisms. Free hemoglobin can also undergo biochemical changes that result in the formation of methemoglobin and release of heme that can further catalyze iron release.[116]

Uptake and degradation of RBCs by macrophages drives heme and iron levels to increase in the cell's cytoplasm working as a signal for cytokine release and iron-mediated generation of reactive oxygen forms that father enhance proinflammatory signals.[1] Intracytoplasmic iron in phagocytes can also result in changes to the cell's gene expression profile so that alternative phagocyte phenotypes that are characteristic of immunosuppression are elicited.[117] This immunosuppressive phenotype would be characterized by higher inducible nitric oxide synthetase expression and therefore couple nitric oxide (NO) regulation to iron homeostasis.[118] In this manner, iron could trigger a cascade of immunosuppressive events that results in inhibition of activating cytokines such as IFN-γ and therefore, inhibit pathways that depend on these active mediators for competent immunological responses.[117] Inhibition of IFN-γ-dependent pathways can also hinder other immune cell types leading to a broader immunosuppressive effect.

MICROPARTICLES AND ADDITIONAL MEDIATORS

Microparticles derived from RBCs are elements that could also play a role in TRIM. RBC-derived microparticles contain hemoglobin and cell-free-hemoglobin that when in the ferrous state can deplete and result in vasoconstriction and inability to vasoregulate (Fig. 6.3).[119] Interestingly, this ability to scavenge NO appears to be more evident when older RBC units are used.[120] Leukoreduction also has limited effect when RBC unit changes are due to storage. This is because reduction in red cell size due to cell membrane losses and increase in rates of nitrite oxidation to nitrate when using

leukoreduced RBC units stored for greater than 25 days may also represent an important element in the storage lesion.[121] Release of ROS by RBCs can also bind NO and potentiate those processes outlined previously.[122] This mechanism may have high relevance, as ROS release by RBC units may be greater in older units.[116,123] Additionally, previously discussed phosphatidylserine is also found on RBC microvesicles that form during storage, resulting in thrombin-dependent complement activation that worsens lipopolysaccharide-induced proinflammatory cytokine production in a mouse model.[124]

Reports have also pointed out that release of ubiquitin by RBCs during storage may also be involved in TRIM. Among cells, ubiquitin is highly expressed in RBCs and just as other mediators its concentration is quantitatively higher in units in a time-dependent manner.[125] Ubiquitin can induce changes in gene expression so that the net result is a Th2 bias in cytokine expression and gene expression.[126] This Th2 activation bias has been the focus of earlier discussion in this chapter. Specifically, exposure to ubiquitin in supernatant from RBC units toward the end of their shelf lives resulted in significant increases in IL-4 and IL-8, while lowering IFN-γ and TNF-α postmitogen stimulation.[126,127]

Biologically active lipids also present in RBC units can also mediate immunoregulation. These lipids such as lysophosphatidylcholine appear to be derived from the remaining plasma in units rather than cells as plasma substitution with buffers significantly reduced their concentration.[128] These lipids can activate neutrophils and this may be one of the factors (first hit) leading to the constellation of symptoms known as transfusion-related acute lung injury (TRALI).[129] Importantly, being plasma-derived would explain why leukoreduction has had limited effect in the reduction of these lipids. Furthermore, as shown in an in vivo transfusion model, the higher the plasma content the greater the concentration of these lipids as seen in immunomodulatory effects of platelet units that have high plasma content.[130] The effect of these lipids will be further covered elsewhere in this book under TRALI.

CELL-DERIVED VESICLES

Cells in the unit release vesicles such as microparticles and ectosomes during storage that increase over time and have been implicated in TRIM. These vesicles are released by aging RBCs,[131] by leukocytes,[132] and by platelets[133,134] with higher concentrations later in a unit's storage life resulting in significant immunomodulatory effects[135]; and in some cases higher mortality in critically ill patients.[136] As most blood cells have been found to release these vesicles, their functions are likely complex with distinct physiology depending on the originating cell lineage. Case in point, platelets have two types of these vesicles, microparticles, and exosomes that have distinct functions not limited to procoagulant activity.[137] On the other hand, those vesicles derived from RBCs and leukocytes may be physiologically immunosuppressive decreasing inflammatory responses.[131,138] They potentially exert some of these functions by direct cell binding or through uptake by leukocytes.[139] Consequently, these vesicular functions reach beyond those in the immune system and include roles in coagulation, vascular homeostasis, and cell development to name a few, and when in excess may increase incidence of adverse outcomes.[140]

ENDOTHELIUM ADHERENCE

One observation that may be of interest is that when RBCs are transfused in an animal model after prolonged storage, leukocytes in the transfused unit lead to higher levels of RBC adherence to capillaries and endothelium as a whole that can be reduced with prestorage leukoreduction.[141] In particular, time-dependent increases in RBC adherence to endothelial cell layers are almost abrogated by prestorage leukoreduction.[142,143] Of interest, poststorage rejuvenation of RBC units, which was covered earlier in the text, also reverses the observed adhesion to endothelial cells.[144] These observations suggest that adherence is secondary to RBC aging while the former places WBCs as the cell group mediating this process. This apparent contradiction will require future research but regardless of the initiating trigger, this adherence is clinically important in the inflammatory dysregulation and binding of RBC to endothelial cells seen in sickle cell disease.[145]

ROLE OF PLATELETS IN TRIM

An increasing number of patients require platelet transfusions, and this number is likely to increase as more patients qualify for hematopoietic stem cell transplantation. Platelet units have in addition to thrombocytes, plasma, leukocytes (remaining postleukoreduction), and RBCs all of which could cause potential complications posttransfusion. Similarly, RBC units also contain platelets that in the preleukoreduction era could cause complex aggregate formation with WBCs leading to a more procoagulable effect and increase the potential for adverse events even with units early in storage.[146,147] Therefore, at this point in the narrative we shift the

focus to platelets and their derivatives as possible mediators of TRIM.

Platelets are not just hemostatic mediators but are essential members of the immune system through their ability to release immunomodulatory cytokines and chemokines, activate neutrophils and form neutrophil extracellular traps, increase expression of endothelial adhesion markers, mediate lymphocyte modulation, leukocyte recruitment, direct killing of infected cells, pathogen sequestration, and direct phagocytosis of invading microorganisms (Fig. 6.1).[148] As a result, platelet transfusions should be seen as an infusion of innate immune hemocytes that will mediate hemostasis, are potentially involved in all arms of the immune response, and in some circumstances be mediators of autoimmunity.[149] Just as in the case of other blood cells, platelets also actively generate microparticles, which are smaller versions of mature platelets that can mediate both activation and suppression of immune responses, and regulate immune cell differentiation.[133,134,150] This immune nature of platelets will likely make them an important participant in responses associated with TRIM and should be the focus of future investigation.

CONCLUSION

Blood component utilization is not without risk, and this can be seen with the 40 years of reports describing TRIM. One think that it is clear that the incidence of TRIM appears to have decreased with the advent of prestorage leukoreduction; nevertheless, as units still have remaining leukocytes, the possibility of TRIM has not fully disappeared. Evidence that TRIM can also be caused by RBCs themselves as they age may signify that these adverse outcomes will prove difficult to avoid. This is further complicated by data showing that soluble mediators found in plasma can also cause TRIM. Furthermore, links of microvesicles, microparticles, and platelets to TRIM implies that a sense of proactive vigilance is needed when using blood. As a result, increased awareness of these responses to transfusion will lead to rapid recognition of these reactions, and implies that a more judicious use of blood components will result in fewer potential cases of TRIM being reported.

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