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Transfusion-related immunomodulation: A reappraisal

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Abstract

Purpose of Review—This review summarizes current and prior observations regarding transfusion-related immunomodulation (TRIM) and puts these ideas into a modern immunological context, incorporating concepts from innate, adaptive, and nutritional immunity. We propose that TRIM research focus on determining whether there are specific, well-defined immunosuppressive effects from transfusing “pure” red blood cells (RBCs) themselves, along with the by-products produced by the stored RBCs as a result of the “storage lesion.” Macrophages are a key cell type involved in physiological and pathological RBC clearance and iron recycling. The plasticity and diversity of macrophages makes these cells potential mediators of immune suppression that could constitute TRIM.

Recent Findings—Recent reports identified the capacity of macrophages and monocytes to exhibit “memory.” Exposure to various stimuli, such as engulfment of apoptotic cells and interactions with β -glucan and lipopolysaccharide, were found to induce epigenetic, metabolic, and functional changes in certain myeloid cells, particularly macrophages and monocytes.

Summary—Macrophages may mediate the immunosuppressive aspects of TRIM that arise as a result of transfused RBCs and their storage lesion induced by-products.

Keywords

Macrophage; RBC transfusion; immunomodulation; innate immunity; memory

Introduction

Transfusion-related immunomodulation (TRIM), originally termed transfusion-induced immunomodulation [1], has been an evocative organizing principle in transfusion medicine for more than 40 years. It is almost always invoked in the context of an “immunosuppression” observable in recipients following transfusion. The Ur-texts in this

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regard were papers by Opelz et al. describing the immunosuppressive effects of prior transfusions in renal transplant patients, demonstrating that multiply transfused patients, who did not develop cytotoxic anti-HLA antibodies, exhibited much better transplant outcomes [2, 3]. This was surprising in that the prevailing wisdom was that blood transfusions had the capacity to induce anti-HLA immune responses, which would either prevent transplantation due to the presence of pre-transplant cytotoxic alloantibodies and/or positive cross-match results, or, for similar reasons, lead to early transplant rejection. Nonetheless, the work of Opelz et al. suggested that non-leukoreduced, packed red blood cell (RBC) transfusions (the standard-of-care at that time) were protective in the era before cyclosporine and other, more effective, immunosuppressive agents [2, 3]. However, even though a positive effect on organ transplantation was documented in the early 1980's, the advent of the AIDS epidemic, a greater appreciation of the prevalence and virulence of "non-A, non-B hepatitis" (i.e., hepatitis C), and the eventual development of modern immunosuppressive agents rendered the practice of deliberate transfusions of donor-specific buffy coats and RBC transfusions to be no longer relevant.

Using the immunosuppressive effects of blood transfusion on transplantation rejection as an analogy, and based on the immune surveillance theory of cancer control, Gantt rapidly hypothesized that these "transfusions might have adverse effects on cancer recurrence and metastasis" [4]. This concept was then explored by Tartter and many others, who published multiple papers supporting this phenomenon in human patients and animal models [5–9]. These results and ideas coalesced around the concept of TRIM, leading to an explosion of studies in human populations and animal models, elaborating the theory explaining a positive effect of "immunosuppressive" blood transfusions in transplantation, autoimmune disorders, and recurrent spontaneous abortions, and a contrasting adverse effect in oncology and infection [10, 11]. This historical literature is well described in multiple excellent reviews [12–14]. However, the expanding primary literature also produced multiple contradictory results leading to a fair amount of controversy, which should be tempered by recognizing that the indications for blood transfusion, along with the methods of blood collection, processing, and storage, have continuously evolved and improved over time.

Text of the review

What transfusion-induced immune effects are NOT defined as TRIM?

To clarify the discussion below, we aim to put these ideas into a modern immunological context, incorporating innate immunity, adaptive immunity, and nutritional immunity [15, 16]. Using this heuristic, the following provides a comprehensive, albeit incomplete, list of potential immunological consequences of blood transfusion that are known to occur in recipients, but *that are NOT classically considered to exemplify TRIM*:

Infectious consequences—Although screening volunteer blood donors for infectious diseases has improved greatly, and although pathogen-inactivation technology has been introduced in several settings, multiple types of transfusion-transmitted infections still occur. As examples, infections with the following etiological agents clearly modulate the immune system of transfusion recipients, but are classically not considered to represent TRIM:

human immunodeficiency virus, human T-cell lymphotropic virus 1/2, cytomegalovirus, and *Plasmodium* species [17, 18].

Plasma-related consequences—Although relatively little plasma remains in modern units of packed RBCs, it is not absent, unless washed or frozen-thawed RBCs are prepared [19]. Thus, various plasma-induced adverse effects can occur, with immunological consequences, although these are not classically considered to represent TRIM. These include allergic/anaphylactic reactions (e.g., in anhaptoalbuminemic or IgA-deficient recipients), transfusion-related acute lung injury, and transfusion-related acute gut injury.

White blood cell (WBC)-related consequences—Although very few granulocytes, lymphocytes, and other WBCs remain in modern, pre-storage leukoreduced, packed RBC units, they are not completely absent. Immunologically-relevant adverse post-transfusion consequences induced by contaminating WBCs include non-hemolytic febrile transfusion reactions, HLA alloimmunization, transfusion-associated graft vs. host disease, and lymphocyte microchimerism, although these are not considered to exemplify TRIM.

RBC-related consequences—Transfusions of leukoreduced, packed RBCs readily induce adaptive immune responses, particularly targeting blood group antigens; these include acute hemolytic transfusion reactions, delayed hemolytic (or serologic) transfusion reactions, autoimmune hemolytic anemia induced following blood group alloimmunization, and prevention of RhD alloimmunization by Rh immune globulin [20, 21]. Interestingly, although the latter is a clear example of (antigen-specific) “immunosuppression,” it is not generally considered to be a type of TRIM. RBC transfusions also affect innate immunity, with specific effects on macrophages, dendritic cells, and granulocytes [22, 23]. Of course, because of crosstalk mechanisms, it is not surprising that effects on innate immunity also influence adaptive immune responses [22, 24]. Finally, RBC transfusions, because of the delivery of the iron present in hemoglobin, influence nutritional immunity by enhancing pathogen virulence [15, 16, 25–27], by producing transfusion-induced iron overload with concomitant effects on immune responses [28], and by potentially reversing the defective immune responses seen in iron-deficiency anemia [29]. Again, none of these immunological effects are classically considered to exemplify TRIM.

Finally, the literature is complicated by the conflation of immunological effects and inflammatory effects, which, admittedly, can often be difficult to separate. For example, many biologically active molecules (e.g., cytokines) affect both the immune response and the inflammatory response. In addition, a given, specific cytokine can be critically important in each of innate, adaptive, and nutritional immunity. Indeed, one could even consider inflammation to be a subset of innate immunity. If nothing else, this implies the need to define, clarify, and identify the responses under study, and to do this specifically, mechanistically, and molecularly.

Focus on TRIM induced by “pure” transfused RBCs

This remainder of this contribution will focus on the occurrence of TRIM following transfusion of RBC-containing products, and will not further consider the literature related

to platelet, granulocyte, or plasma transfusions. Nonetheless, even this narrowly defined approach is complicated by the availability of multiple different product types containing autologous or allogeneic RBCs, including whole blood, packed RBCs, leukoreduced (by multiple methods) packed RBCs, washed RBCs, frozen-thawed RBCs, pathogen-inactivated RBCs, irradiated RBCs, and RBCs transfused after intra-operative salvage. In addition, several different collection methods (e.g., whole blood and apheresis) and multiple storage solutions are used (e.g., CPDA-1, AS-3, SAGM). Finally, transfusion practices have evolved over the last 40 years and continue to evolve. These variations make it difficult to compare results obtained in various studies, particularly when evaluating those completed 20-40 years ago.

For these reasons, and with a goal of looking towards the future, the remainder of this review will focus only on the effects of transfusions of units of pre-storage, filter-leukoreduced packed RBCs; this is the predominant, current, standard-of-care product used in most countries. Nonetheless, these units do contain small amounts of plasma and platelets, and very small numbers of WBCs (predominantly lymphocytes), along with storage solution and storage-induced components, any and all of which could affect the recipient's immune system.

A proposed definition of TRIM, accompanied by caveats

If all of the above immunologically relevant consequences of RBC transfusion are excluded from the definition of TRIM, what is left? Alternatively, is the original definition of TRIM so nebulous and all-encompassing that it is virtually meaningless? Finally, are the methods used to identify the presence of TRIM (e.g., human studies quantifying global outcomes, such as cancer progression and/or tumor metastasis in transfusion recipients) too broad and superficial to yield mechanistic understanding? To address these concerns, we believe that a more specific, more defined, epidemiological definition should lead to improved molecular-based understanding of the underlying mechanisms, thereby facilitating our ability to exploit or ameliorate these effects.

Thus, we propose that:

The concept of TRIM focus on determining whether there are specific, well-defined immunosuppressive effects from transfusing “pure” RBCs themselves, along with the by-products produced from the stored RBCs as a result of the “storage lesion.”

The latter “by-products” include constituents of the storage solution, which contains bioactive molecules such as adenosine (which can be removed by washing), and storage-induced by-products of the RBCs themselves, including lactate, oxidized lipids, microvesicles, hemoglobin, heme, and “free” iron. Nonetheless, there are already known effects of these particular oxidized lipids on inflammation, of heme on TLR4-linked inflammatory pathways, and of iron on nutritional immunity. Thus, even with this rather narrow definition, it remains confusing which of these specific effects, if any, should be included in our understanding of TRIM. Indeed, it is fair to ask whether including multiple, highly-specific findings into an all-encompassing concept of TRIM adds any value. In addition, it is important to remain cognizant that modern, leukoreduced, packed RBC units do contain some plasma contamination (which can be removed by washing), some WBC

contamination (which can be removed by washing and inactivated by irradiation), and variable amounts of contaminating platelets (which are not completely removed by leukoreduction filters).

Finally, the “downstream” consequences need to be clearly and specifically defined. For example, when studying cancer, the underlying mechanisms promoting local recurrence may differ significantly from those responsible for distant metastases. In addition, neoplasia in different patients, even of “identical” pathological type, can differ significantly and demonstrate significant intra-tumoral heterogeneity. Alternatively, when studying infectious diseases, the promotion of infection may markedly differ for intracellular, as compared to extracellular, pathogens. In addition, transfusion effects on “ferrophilic,” as compared to non-ferrophilic, pathogens can differ significantly. Finally, even for a defined genus and species, strain-specific differences can affect virulence and sensitivity to host immunity.

Given these caveats, when studying TRIM, it is also important for pathophysiological understanding and practical considerations to determine whether the effect under study is produced following autologous and/or allogeneic transfusion, and whether it occurs following transfusion of fresh (i.e., collected, but not stored) and/or stored RBCs.

Some of these issues are well illustrated in a carefully described rat model [30], which compared fresh and stored RBC transfusions, both filter-leukoreduced and non-leukoreduced, from both allogeneic and syngeneic donors. The outcome measures included a model of blood-borne metastasis using an adenocarcinoma cell line (although short-term pulmonary “retention” of infused tumor cells was measured, rather than actual metastasis) and a model of local tumor growth and enhanced tumor burden using a leukemia cell line. In their carefully controlled experiments, transfusions of stored, but not fresh, RBCs induced TRIM effects and these effects were produced equally by allogeneic and syngeneic (e.g., “autologous”) RBCs. In addition, the observable TRIM effects were due to the RBCs themselves, not to contaminating WBCs or to products of the storage lesion contained in the stored RBC supernatant. Although these phenomenological results were very clean, they could not rule out a role for contaminating platelets, which were probably present in their filter-leukoreduced products. In addition, although they hypothesized that the mechanisms underlying these results were due to classical immunological mechanisms, such as those involving T, B, and/or NK cells, other explanations are possible. For example, it is plausible that circulating non-transferrin bound iron (NTBI), which is produced in animal and human recipients following stored RBC transfusions [31–33], could cause endothelial cell damage [34–36], thereby increasing capillary permeability and enhancing endothelial cell adhesion molecule expression [37, 38]; thus, this effect on nutritional immunity could conceivably enhance circulating tumor cell “retention” in the pulmonary capillary bed. Alternatively, if this mechanism were true, one could conceivably propose that the effects of NTBI had nothing to do with nutritional immunity and, instead, were simply due to its direct toxic effects on endothelial cells. Finally, because iron functions as a growth factor for neoplastic cells [39], the provision of iron from a stored RBC transfusion could simply provide “nutrition” to the tumor, without needing to implicate the immune system.

A possible way forward

Despite the caveats and problems described above, we propose, as one way forward, that useful information might be gained by focusing on the “immunosuppressive” effects that “pure” RBC transfusions may have on specific aspects of the innate immune system, particularly on macrophage and monocyte function. These potential effects can be subdivided into acute, sub-acute (i.e., intermediate), and chronic (i.e., long-term) categories.

Acute effects—The initial interaction between the innate immune system and storage-damaged, transfused RBCs involves phagocytosis, primarily by macrophages, but also by monocytes and, to a much lesser extent, by dendritic cells and granulocytes in mouse models of transfused, storage-damaged RBC clearance. Erythrophagocytosis, followed by lysosomal catabolism of the ingested RBCs, can rapidly produce NTBI [31–33], induce the release of specific cytokines [31], inhibit subsequent macrophage function, and damage lysosomes, potentially leading to cell death [40, 41]. In this context, macrophage cell death may have an “immunosuppressive” effect on innate immunity by decreasing the number of available phagocytes. However, robust erythrophagocytosis can also promote an inflammatory response with increased expression of circulating cytokines [31]. In addition, NTBI, in the context of nutritional immunity, could enhance pathogen virulence [16]. Taken together, these outcomes could produce a TRIM effect of enhancing post-surgical or nosocomial bacterial infection rates and severity [42]. In addition, ingestion of (antibody-coated) RBCs by splenic granulocytes [23] can impair oxidative-burst function [41], thereby potentially impairing this type of protection against invading blood-borne pathogens.

Sub-acute effects—Macrophages exhibit substantial plasticity and diversity, enabling them to change their phenotype rapidly in response to select stimuli. Several macrophage polarization states have been described that extend beyond the M1 and M2 distinctions, characteristically reserved for “classically” or “alternatively” activated macrophages, respectively. These include other subgroups [43, 44] in response to additional stimuli, such as to oxidized phospholipids (i.e., Mox) [45, 46], and the CXCL4 chemokine (i.e., M4) [47, 48]. One polarization state that may be particularly relevant to TRIM is Mhem, which arises from macrophage stimulation by heme and hemoglobin. Though currently primarily studied in atherosclerotic plaques, Mhem macrophages are defined by increased heme oxygenase-1 expression accompanied by anti-inflammatory phenotypic properties [49–53]. All of these various polarization states are intimately interrelated to particular metabolic pathways (i.e., “immunometabolism”) [54, 55], and it is conceivable that erythrophagocytosis can induce changes in the functional polarization of the ingesting macrophage and/or its neighboring cells [56]. These effects would be expected to take place over a period of days, and could affect subsequent responses to exposure to pathogens or transfused RBCs, or affect the immune surveillance of neoplastic cells.

Chronic effects—An additional concept relating to monocyte and macrophage functional plasticity has been discovered recently; that is, these key players in the innate immune system can exhibit “memory,” a characteristic previously thought to be limited to lymphocytes involved in adaptive immunity. To this end, monocytes and macrophages that are exposed to certain stimuli can develop metabolic and epigenetic changes that lead to

long-term alterations in subsequent responses by these cells. To further highlight their versatility, the memory established in monocytes and macrophages can be stimulatory or suppressive, with both effects often occurring simultaneously, depending on which genes are expressed or suppressed [57]. The process of macrophage “priming” or “training” occurs when prior exposure to a specific stimulus induces an enhanced immune response, such as increased microbicidal activity (i.e., increased phagocytic activity and increased expression of pro-inflammatory genes) [58]. As examples, stimuli that trigger priming can include phagocytosis of apoptotic cells [59], ingestion of β -glucan (present in the *Candida* cell wall) [60], or exposure to lipopolysaccharide (LPS) [61]. In addition to altering gene expression, these stimuli can induce changes in metabolic function, such as triggering macrophages to switch to glucose metabolism to increase energy production for supporting increased cellular activation [62]. In contrast, “tolerance” or “immunoparalysis” occurs when prior exposure of macrophages to a specific stimulus results in decreased responses to subsequent exposures to that stimulus or similar stimuli; LPS is the most-studied example of such a tolerizing response in macrophages [60]. Interestingly, these memory-inducing events can also interact with each other; for example, “priming” exposure to fungal β -glucan can reverse the tolerizing effects of LPS-induced immunoparalysis [63].

The stimuli to which monocytes or macrophages are exposed can widely vary based on their location within an organism. In the context of possible RBC transfusion-mediated metabolic reprogramming, splenic red pulp macrophages and hepatic Kupffer cells are likely targets, given their physiological roles in erythrophagocytosis and iron recycling. For example, enhancer landscapes of murine splenic macrophages and Kupffer cells were found to cluster together, presumably because they share similar microenvironments with substantial physiological exposure to senescent RBCs [64]. In this context, the contents of RBCs (e.g., heme, iron, and hemoglobin) may function as tolerance-inducing stimuli. In pathological settings, such as the transfusion of storage-damaged RBCs or during IgG-mediated transfusion reactions, these cell types clear substantial amounts of RBCs in a relatively short period of time, which contrasts with their normal physiological roles. This suggests the testable hypothesis of whether pathologically increased erythrophagocytosis can “train” macrophages and monocytes. In the context of TRIM, one hypothesis would be whether increased erythrophagocytosis by macrophages or monocytes induces epigenetic and/or metabolic “immunosuppressive” alterations that affect their ability to function appropriately in response to subsequent stimuli, such as exposure to invading pathogens [65], inflammatory disorders, surgery, trauma, or immune surveillance of neoplastic cells. In addition, it is possible that the effects on macrophages and monocytes may not only be mediated by the ingested RBCs *per se*, but also by the signal transduction pathways activated by the relevant cell surface receptor recognizing the “damaged” RBC (e.g., various Fc-gamma receptors for IgG-opsonized RBCs).

Conclusions

The overarching concept of TRIM has inspired a great deal of important research over more than 40 years, producing both enlightenment and confusion. Depending on one’s point of view, TRIM can explain many of the immune effects of blood transfusion, or it can have relatively little pathophysiological relevance. By focusing the definition of TRIM on the

immunosuppressive effects of RBC transfusions on innate immunity, particularly on macrophage/monocyte function, additional discoveries are possible. Nonetheless, we recommend targeting the molecular and cellular mechanisms underlying disease-specific and molecularly-defined outcomes.

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Key Points

- Blood transfusions can have immunological consequences that are not classically considered to exemplify transfusion-related immunomodulation (TRIM); these can be mediated by transfusion-transmitted infections or by various blood components in the transfusion product, including plasma, white blood cells, and red blood cells (RBCs).
- We propose that the definition of TRIM focus on determining whether there are specific, well-defined immunosuppressive effects from transfusing “pure” RBCs themselves, along with the by-products produced by the stored RBCs as a result of the “storage lesion.”
- Macrophages, a key cell population involved in erythrophagocytosis, may play an important role in mediating the immunomodulation induced by transfused RBCs.
- RBC phagocytosis by macrophages has the potential to produce acute, sub-acute, and chronic effects on macrophage function.