Regulation of immune responses by RBC transfusion

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It has long been appreciated that transferring allogeneic tissues between members of the same species can have complicated effects upon the recipient immune system. Transfusion represents the most common exposure to large quantities of alloantigen, which unlike solid organ transplant, occurs in the absence of pharmacological immunosuppression. Transfused products constitute a complex cellular entity, with multiple potential pathways of affecting recipient immunity. In many ways, transfusion is a complex and variable cellular therapy, the broad effects of which are poorly understood. In addition to the well-described phenomenon of direct alloimmunization, multiple transfusion induced alterations of the immune response to third party alloantigens or to antigens in general have also been described, the biology of which has been named ‘Transfusion Related Immunomodulation (TRIM)’. Early observations of TRIM effects most famously included the striking observation that transfusing red blood cells (RBCs) prior to renal transplant, substantially delayed organ rejection. This phenomenon, which was named ‘The Transfusion Effect’, was interpreted by some as evidence that transfusion tolerized to alloantigen, whereas others regarded this as a reflection of a general immunosuppressant property of transfused blood. This latter interpretation was bolstered by subsequent observations that increased frequency of transfusion correlated with increased rates of infection, neoplastic metastases, and decreases in symptoms of autoimmune disorders. In aggregate, the above observations have led to a general belief, across multiple fields and medical specialties, that transfused blood products are immunosuppressive. However, such understanding has evolved in the context of the clear demonstration that transfused blood can induce a primary immune response in the form of alloantibodies. This leads to the apparently paradoxical landscape of transfusions being simultaneously ‘immunosuppressive’ and ‘immunogenic’. The proposed solution to the apparently conflicting observations is a widely stated paradigm that transfusions stimulate humoral immunity whilst simultaneously inhibiting cellular immunity. Such could certainly be the case, and is consistent with more general understandings of mutually antagonistic arms of the immune system (e.g. Th1 that favors cellular immunity and Th2 that favors humoral immunity). However, it must be emphasized in the strongest possible terms that the vast majority of studies that describe TRIM effects do not in fact measure immunity. Rather, they measure biological outcomes that may be affected by immunity, but may just as likely be affected by processes independent of the immune system. In support of this notion, recent reports have indicated that transfused blood can have growth factors that directly stimulate bacterial growth and tumor expansion, bringing previous interpretations of immunosuppression, based upon infections and metastasis, into question. However, recent studies on TRIM effects have indeed focused on innate immunity and inflammation, and in doing so have started to tackle the direct issue of measuring the immune response itself; however, the absence of...
It has long been appreciated that transferring allogeneic tissues between members of the same species can have complicated effects upon the recipient immune system. Most obvious is the induction of alloimmunity that can result in rejection of the transplanted tissues. However, of equal importance, if not more subtle effect, are alterations of the immune response to third party alloantigens or to antigens in general. These complicated immunological landscapes have been observed in solid organ transplantation, in bone marrow transplantation and during allogeneic transfusion. However, the nature of such effects differs profoundly, at least in part, as a function of the nature of the transplanted tissue.

Our vision of immunoregulatory effects during solid organ transplantation is substantially blurred by alterations to immunity through the pharmacological immunosuppressants that are required to prevent organ transplant rejection. Early hints at such effects were observed prior to the advent of potent immunosuppressant drugs, as in the observation that kidneys transplanted between pigs were almost uniformly rejected, but rejection was prevented if liver tissue was transplanted prior to renal transplant. However, in large part, such studies (and certainly translation of such approaches into humans) have not progressed due to the overwhelming success of pharmacological immunosuppression. Ironically, these early observations have inspired ongoing efforts to develop allotolerance and thus avoid the need for chronic immunosuppression; however, such approaches are typically carried out by targeted immunomodulation strategies involving engineered cellular therapies and related approaches as opposed to transfusion.

Unlike solid organs, the vast majority of bone marrow transplantation (BMT) does not require immunosuppressants to prevent rejection. This is likely due to the fact that most BMT is performed on patients being treated for neoplasia. Thus, the conditioning regimens utilized virtually eliminate the recipient immune system. In such a case, immunosuppressants may be required to prevent or mitigate graft versus host disease. However, study of the effects of bone marrow on recipient immunology is a non-starter, as the recipient immune system is gone.

In contrast to the above scenarios, transfusion results in exposure to alloantigens with neither pharmacological immunosuppression nor chemotherapeutic ablation of the recipient immune system. This is not to say that the immune system of transfusion recipients is necessarily normal; indeed, the underlying pathology for which the patient is transfused and/or drugs used for treating said pathology may alter immunity substantially. Nevertheless, in many transfusion recipients, alloantigens are infused in the context of an intact immune system without immunosuppressant drugs. This provides a unique landscape to study basic processes of immunomodulation through introduction of complex cellular mixtures of alloantigens, the biology of which has been dubbed ‘Transfusion Related Immunomodulation (TRIM)’ [1]. As these observations are occurring in the context of ongoing standards of patient care, the underlying findings have substantial ramifications for both potential medical sequelae and/or additional therapeutic benefits other than the primary goal of transfusion to repair a deficiency in circulating blood cells.

Early observations of TRIM effects most famously included the striking observation that transfusing RBCs prior to renal transplant, substantially delayed organ rejection. This phenomenon, which was dubbed ‘The Transfusion Effect’, was interpreted by some as evidence that transfusion tolerized to alloantigen, whereas others regarded this as a reflection of a general immunosuppressive property of transfused blood [2, 3]. This latter interpretation was bolstered by subsequent observations that increased frequency of transfusion correlated with increased rates of infection, neoplastic metastases and decreases in symptoms of autoimmune disorders [1]. Moreover, transfusion correlates with a decrease in recurrent pregnancy loss, which has been given an immunological interpretation. In aggregate, the above observations have lead to a general belief, across multiple fields and medical specialties that transfused blood products are immunosuppressive. However, such understanding has evolved in the context of the clear demonstration that transfused blood can induce a primary immune response in the form of alloantibodies. This leads to the apparently paradoxical landscape of transfusions being simultaneously ‘immunosuppressive’ and ‘immunogenic’. The proposed solution to the apparently conflicting observations is a widely stated paradigm that transfusions stimulate humoral immunity whilst simultaneously inhibiting cellular immunity. Such could certainly be the case, and lines up with more general understandings of mutually antagonistic arms of the immune system (e.g. Th1 that favours cellular immunity and Th2 that favours humoral immunity). Such a hypothesis may hold widespread explanatory power, despite some evidence in animal models that transfusion can induce cellular immunity.
at the same time as humoral immunity [4–7]. Although not evidence in of itself of a hypothesis’s validity, due to errors of affirming the consequent, this general understanding nevertheless does conform to the widespread and numerous observations referred to above.

The above history and observations generate a complicated intellectual landscape for several reasons, and careful thought must be given to the conclusions that have been drawn, and thus serve as the basis for ongoing study. First and foremost, it must be emphasized in the strongest possible terms that the vast majority of studies that confirm TRIM effects do not in fact measure immunity. Rather, they measure biological outcomes that may be affected by immunity, but may just as likely be affected by processes independent of the immune system. A clear example of this is to be found in the multiple studies looking at the effect of transfusion on infectious disease complications in the transfusion recipient [1]. To the extent that the correlations between transfusion and increased infectious disease are statistically significant, and assuming that they are not the result of an indication bias or other confounder, then such an observation is of extreme medical importance. Speculations as to a causal relationship, rather than just correlative, inevitably leads to the generation of hypotheses regarding mechanism of action; indeed, in these contexts, the prevailing hypothesis has been that transfusion suppresses immune function leading to increased infectious disease by limiting host immunity. Indeed, such an interpretation has seemed so obvious, to the exclusion of other notions, that many have formulated the unequivocal conclusion that transfusion is immunosuppressive. However, as above, this conclusion is reached without ever measuring the immune system. In some cases, in vitro assays will examine very blunt measures of general lymphocyte function (i.e. proliferation induced by non-specific stimuli such as phytohemagglutinin (PHA) or lipopolysaccharide (LPS)), but these studies do not reflect the in vivo complexities of an authentic immune response. Along these lines, there is typically no investigation, whatsoever, of either innate or adaptive immunity (i.e. antibody or effector cellular response) to support such conclusions. This becomes problematic in the context of alternate hypotheses that are equally consistent with the data. For example, the data equally support a hypothesis where transfused blood products contain growth factors that promote microbial growth. Indeed, it has recently been reported (in an animal model) that the iron contained in stored RBC units serves as a growth factor for ferrophilic bacteria, with supportive data from human studies [8–11]. These findings in no way challenge the base observation that rates of infection are increased after transfusion; indeed, they provide supportive evidence and potential mechanisms. However, as this mechanism does not involve immunosuppression, these findings potently illustrate a fundamental problem that can be found in the interpretations across the TRIM literature.

Recently, in an attempt to further understanding of TRIM biology, several groups have begun to analyse effects of transfusion on immune function in whole animal systems, using both model animals (e.g. mice and dogs) and also in human studies. These studies have focused mostly on activation of innate immunity. In collaboration with several groups, we have analysed the effects of stored RBCs on regulation of innate immunity post-transfusion. A substantial advance was made by Hod et al. using a murine system of RBC storage and transfusion, which demonstrated that transfusion of stored RBCs resulted in a burst of recipient serum inflammatory cytokines [11]. Moreover, if the recipients were primed with a model of endotoxemia, transfusion of stored RBCs caused a greatly augmented cytokine response that resulted in severe illness in the recipient animal. In neither case did fresh RBCs have any observed negative effects on the recipient (either with regards to cytokines or general constitution). The inflammatory substance(s) in this case are both associated with the RBC and require the RBC to be intact, as neither supernatants nor whole lysates of the RBC unit had an effect upon transfusion. Follow-up studies have shown a similar response in canine recipients [12].

A controlled human trial using healthy volunteers did not show a similar cytokine response, or any measurable cytokine response at all for the measured entities, upon transfusing a single unit of 42 day old autologous RBCs [8]. Thus, this represents a very important pertinent negative, and to the extent that the 14 subjects reflect general human biology, then there seems to be little detectable effect of stored RBCs on the indicators of innate immunity that were measured in healthy recipients. However, reflection on the data reported in the mouse studies demonstrates that only small responses were seen in mice that received a single unit. A recent study on two units of cryopreserved RBCs transfused into humans also failed to detect a cytokine response; however, these did not constitute ‘old’ RBCs as they were frozen fresh [13].

Of great importance is that responses were amplified tremendously in mice primed with endotoxin, and the above human studies were in healthy adults. Thus, it remains formally largely unknown if a similar biology occurs in sick humans. However, recent reports indicate that transfusing RBCs into sick humans may induce cytokine responses in a way analogous to that predicted by the animal models [14, 15]. Thus, additional study is clearly required in this area; nevertheless, it has been unequivocally observed in mice and dogs, that stored RBCs have a profound effect upon innate immune activation – an observation that has been recapitulated in some human studies. Moreover, analysis of
a large cohort of human intensive care unit patients has reported alterations in multiple cytokine parameters after transfusion, in some cases lasting for prolonged times [16].

It is in the above context that our research group, along with our collaborators, has focused on alterations in innate immune responses as a result of transfused blood products. We have reported not only an acute alteration in serum cytokines but also have analysed characteristics and components of the blood units that may be mechanistically involved. Multiple sources of damage to RBCs (e.g. storage, heat damage, pharmacological oxidation), each of which results in rapid clearance upon transfusion, all result in a similar cytokine storm in recipient mice. Thus, it seems likely that it is the rapid consumption of RBCs that is linked to innate immune activation. We have also observed that murine donor units generate substantial volumes of leukotrienes and prostaglandins during storage, as a result of release of arachidonic acid from cellular membranes and subsequent conversion by cyclooxygenase and lipooxygenase. This precise same chemistry has been observed in multiple human units; however, the effect remains unclear [17]. In the murine system, higher levels of these bioactive lipids correlate with blood that is more inflammatory upon transfusion. Thus, in aggregate, substantial evidence is accumulating that stored blood products accumulate damage that may induce recipient innate immune alterations, by multiple mechanisms. This trend gets at the more immediate heart of the matter of TRIM studies, in particular, how does transfusion affect immunity itself as opposed to surrogate measures.

In summary, much more remains unknown than is known at the current time regarding the effect of transfusion on immune function. Tremendous amounts of retrospective clinical evidence suggest an effect in humans [1]. Accumulating basic science evidence demonstrates an unequivocal effect in mice and dogs, and potential mechanisms of said effects are beginning to emerge. However, the field has yet to bridge the divide between the animal studies and an understanding of the human biology. Much study remains to be done; indeed, one could argue it has yet to be firmly established that a robust phenomenology in humans even exists.

Disclosure

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