Editorial Commentary

Refractoriness to platelet transfusion in the presence of anti-HLA antibodies—reassessing the alloantibody hypothesis

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Since platelet products first became routinely available in the 1970s, platelet transfusions have been a mainstay of the treatment of thrombocytopenic patients, decreasing the frequency and severity of bleeding sequelae (1). While the overall clinical goal is to decrease bleeding, the success of a platelet transfusion is often determined by an increase in platelet count post-transfusion [often reported as corrected count increment (CCI)] (2). The CCI may range from a substantial increase in platelet count to no effect at all; in some cases, a decrease in CCI can even be observed post-transfusion. Inadequate CCIs are to be expected by chance alone with some frequency, on a unit-by-unit basis, given the significant donor variability in platelet quality. However, some patients consistently have unusually low CCIs despite transfusion of multiple platelet units from different donors—such patients are designated as "refractory" (1). While substantial progress has been made in recent decades in understanding different causes of refractoriness, treating thrombocytopenia in refractory patients remains one of the major challenges in platelet transfusion therapy.

Humoral alloimmunization is considered to be a major cause of refractoriness, estimated to be responsible for approximately 20% of cases (1). The prevailing theory is that patients develop alloantibodies against alloantigens on platelets [e.g., Human Leukocyte Antigens (HLA) or Human Platelet Antigens (HPA)] (1). Such alloantibodies presumably occur in response to exposure to the alloantigen(s) during transfusion therapy and/or through antecedent exposure from pregnancy or transplantation. Once formed, alloantibodies bind to alloantigen(s) expressed on donor platelets and cause rapid clearance (called the alloantibody hypothesis herein). This theory is widely accepted in the field and it is justified by substantial empirical evidence (1,3,4). Moreover, once a patient is designated as refractory for immunological reasons, the administration of HLA matched platelets achieves greater CCIs than random donor platelets (5-7). Data in canine and murine models provide additional evidence that alloantibodies can be responsible for clearance of transfused platelets (8-11). Thus, there are deducible consequences of the alloantibody hypothesis that are observed both in humans and experimental animals.

Numerous studies including the landmark Trial to Reduce Alloimmunization to Platelets (TRAP) (4) have noted that a substantial number of patients who develop alloantibodies do not become refractory. This represents a large practical problem in patient management, both in terms of predicting which patients may become refractory, and also in determining if alloantibodies are the cause of poor CCIs (as opposed to other reasons). As such, the field has focused on identifying characteristics to distinguish refractory causing alloantibodies (RCAs) from alloantibodies that do not cause refractoriness (non-RCAs). Archived samples from the TRAP trial (12,13), as well as new samples collected over time (14-18), have been analyzed in search of properties that distinguish RCAs from

non-RCAs. A large number alloantibody characteristics have been considered, including titer of antibody (19), IgG subtype (20), post-translational modifications (21), and the specific HLA being recognized (15). Although a correlation with titer and refractoriness has been reported in one study, none of the above approaches have revealed a clear answer. The methods of detecting alloantibodies and the cutoff for being "positive" have also been scrutinized (12), but has led to little clarity. Thus, as we reach 25 years of work since the TRAP trial, it remains entirely unclear what distinguishes RCAs from non-RCAs. However, the search for a defining characteristic of RCAs continues as new and more sophisticated tools of biochemistry and cell biology develop and as theory of alloantibodies and platelets continue to mature.

In a highly innovative and rigorous report, Rijkers *et al.* have advanced our mechanistic understanding of alloantibody-platelet interactions (15). They demonstrated that a subset of anti-HLA alloantibodies can cause activation of donor platelets and phagocytosis by macrophages *in vitro* through a mechanism involving FcγRIIa crosslinking (15). This resembles similar pathways identified in immune thrombocytopenic purpura (ITP) (22) and transfusion-related acute lung injury (TRALI) (23). Rijkers *et al.* proceeded to show that alloantibodies specific for the same HLA molecule have different effects on platelet activation (15). The mechanisms are unclear, but the data from Rijkers *et al.* suggested that epitope recognition, rather than affinity, determines the alloantibodies' effects (15). This group has also reported that geometry of antibody binding affects how complement is activated (16).

While these findings are of substantial importance in understanding mechanisms of alloantibody interaction with platelets, at least from our point of view, the data in hand seem to argue against the utility in predicting RCAs *vs.* non-RCAs. For example, only 30% of patient sera activated donor platelets *in vitro* despite all patients being refractory with detectable anti-HLA alloantibodies (15). It remains possible that the frequency of activating alloantibodies is higher in refractory than non-refractory patients since only sera of patients known to be refractory with anti-HLA alloantibodies were tested. Thus, it is unclear if alloantibody induced platelet activation truly plays a role in refractoriness. If it does, activation of donor platelets by recipient sera should predict the CCI for a given platelet unit. This remains to be tested.

After several decades of effort to identify what distinguishes RCAs from non-RCAs, it seems fair to ask why has so little progress been made. This is not to say that new understanding about alloantibodies has not been generated—Beligaswatte *et al.* have noted that quantity of alloantibodies as measured by intensity of HLA-coated bead binding correlates with refractoriness (19). However this has yet to be validated in a larger clinical context and is in apparent disagreement with other studies (15). While most guidelines recommend testing of anti-HLA antibodies in patients with serial inadequate CCIs, there is no standard for the particular assay or its interpretation (2). Thus, practically speaking, we remain dependent on CCIs alone to determine refractoriness and continue to lack an ability to predict *a priori*. Of course, there could be some characteristic of RCAs that is different from non-RCAs and deeper and deeper characterization over time will eventually uncover the answer. However, it may also be time to take a step back and re-assess the supposition that there must be a difference between RCAs and non-RCAs that explains why some alloimmunized patients are refractory and others are not.

There are at least two different ways in which some patients with alloantibodies can be refractory, and others not, but with no difference in the alloantibodies between these two groups. First, alloantibodies may be necessary, but not sufficient for immune mediated refractoriness. Alloantibody mediated platelet clearance likely requires *in vivo* biology that is not present in a serum/plasma sample (e.g., the reticuloendothelial system, vascular flow through capillary beds certain organs, etc.). Recipient genetic polymorphisms (such as in Fcγ receptors and the complement system) may determine refractoriness in a patient with alloantibodies. If true, the answer would never come from analyzing alloantibodies. Moreover, transfusion medicine faces the challenge of wide genetic diversity in both the patient population and the therapy itself. Indeed, Rijkers *et al.* showed that the platelets from different donors activated differently in response to the same alloantibody (15). This finding is consistent with known variability of platelets from donor to donor in other assays measuring platelet activation in response to antibody binding. For example, the performance of serotonin release assays for heparin induced thrombocytopenia depends upon the use of specific platelet donors (24). The search for predictive diagnostics may have to extend beyond plasma.

Second, the reason a defining characteristic of RCAs remains elusive may be the validity of the alloantibody hypothesis itself. Given that antibodies can clear numerous biological targets, it is reasonable to assume that alloantibodies clear platelets. Yet, a large number of patients

have anti-HLA alloantibodies and are not refractory (4). This could be reconciled by searching for additional characteristics of alloantibodies, or alternatively, considering a new hypothesis. That said, an outright rejection of the alloantibody hypothesis would cause its own problems including the loss of an explanation of the efficacy of HLA matched platelets in refractory patients with alloantibodies. The challenge is to consider if there are any alternative hypotheses that would predict all of the available data. One such hypothesis is that CD8+ T cells cause platelet refractoriness.

Alloantibodies and CD8+ T cell responses tend to go hand in hand. While there certainly can be a pull and tug between the arms of the immune system (analogous to Th1/Th2 paradigms in mice), the issue is usually the nature rather than the absence of antibody. As such, anti-HLA alloantibodies may be indicators of immunity and correlate with CD8+ T cell mediated platelet clearance. The role of CD8+ T cells has yet to be assessed in platelet refractoriness, as there are no clinical laboratory assays that test CD8+ T cell function regarding platelet targets. However, there is evidence to support the CD8+ T cell hypothesis. In a mouse model after alloimmunization, CD8+ T cells cause platelet refractoriness in the absence of any alloantibodies (25). Whether this translates into humans has yet to be established. We are not advocating for rejecting the alloantibody hypothesis nor are we advocating for the CD8+ T cell hypothesis in particular. However, at the very least, we present the CD8+ T cell hypothesis as an example of an alternative theory to the alloantibody hypothesis that is equally consistent with the known data. The CD8+ T cell hypothesis is not in conflict with failure to identify defining characteristics of RCAs and still explains efficacy of HLA matched platelets in refractory patients. Of course, other theories in addition to alloantibodies or CD8+ T cells can be put forth that likewise explain the data, but space limitations preclude their presentation and discussion here. In light of difficulties defining RCAs, consideration of alternative theories is warranted while simultaneously refining alloantibody characterization.

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