Severe reactions associated with transfusion of patients with sickle cell disease

Every immunohematologist or physician who has had to participate in the transfusion of patients with sickle cell disease (SCD) knows that there may be many problems to overcome. Most of the emphasis in the immunohematology literature has been on alloimmunization. In 12 reports, alloimmunization occurred in 8 to 35 percent (mean, 25%; median, 25%) of 2818 transfused SCD patients.[1-12] Anti-E (21%), -C (14%), and -K (14%) were, by far, the most common antibodies encountered. Anti-Fy^a (7%), -Jk^b (5%), -S (4%), and -D (4%) were the next most common antibodies of possible clinical significance. Lewis system antibodies were also common (11%), but it was unclear from the reports whether they were stimulated by the transfusions or were present before transfusion.

Although there are in the literature many reports[7,13-26] with detailed case histories of hemolytic transfusion reactions associated with SCD, their incidence in SCD is not so well documented. Delayed hemolytic transfusion reactions (DHTRs) in transfused SCD patients have been reported in four series to have an incidence of 4, 11, 17, and 22 percent.[6,7,9,14] There is no doubt that the incidence of DHTRs in SCD is much higher than that reported for random patients who received transfusions, which has been reported to be one DHTR per 854 to 2537 patients.[27-29]

Most of the reports of DHTRs in SCD follow the typical pattern of a DHTR, with new alloantibodies (Rh system, mainly anti-C and -E [39%], -Jk^b [15%], -Fy^a [10%], -Jk^a and -S [7% each], -K, -Fy^b, and -s [3% each]) appearing after 7 to 10 days, obvious signs (laboratory and clinical) of hemolytic anemia, a positive direct antiglobulin test, and decreased survival of the transfused red cells (RBCs). Many of the reports[7,13-17,19,20-26] contain findings that are not typical of DHTR: pain crises (87%), posttransfusion hemoglobin or hematocrit dropping below pretransfusion levels (83%), hemoglobinuria and/or hemoglobinemia (33%), negative direct antiglobulin test (26%), pulmonary infiltrates (9%), disseminated intravascular coagulation (7%), and new RBC alloantibodies that were not detectable until ≥72 hours after the DHTR (7%) or that were never detected (20%). Some patients with SCD and DHTRs develop a life-threatening or fatal (11%) anemia with some, or all, of the unusual findings above. This phenomenon was first reported in an organized way by Diamond et al.,[14] from the National Institutes of Health, who described four patients with DHTRs presenting as sickle cell crises. All of their patients were treated for sickle cell pain crisis before the association with a DHTR was noticed. The authors emphasized that, in the future, they would evaluate all SCD patients who have painful posttransfusion crises for DHTRs. Other authors[2,7,15,16,18,21-26] agreed with and have extended the findings of Diamond et al.[14] Milner et al.[16] described a posttransfusion syndrome involving fever, bone pain, bone marrow infarcts, serous effusions, hypertension, seizures, and cerebral hemorrhage.

Controversy continues over the cause of the acute life-threatening anemia, termed "hyperhemolysis" by some investigators,[22,23] that occurs after transfusion in patients with SCD. It is easy to show, by measuring hemoglobin A and S, that the donor RBCs often are completely destroyed. The reason for this is sometimes obvious when new antibodies appear and the transfused units possess the putative antigens, and sometimes the pertinent alloantibody may be eluted from any remaining RBCs. However, in 27 percent of the cases reported above,[13-26] the alloantibodies were not detectable at the time of the DHTR, but become detectable later, or were never detected. Chaplin and Cassell[30] reported a detailed study of an SCD patient who had many DHTRs with sickle cell pain crises, but in whom no antibodies were detected. The only normal RBC survival was noted when a unit of blood from the patient's sister was transfused; 1 year later, the sister's RBCs survived normally for 5 to 7 days and then disappeared rapidly from the circulation. At this time, the patient was seen to have a typical painful sickle cell crisis. We recently described 70 cases of hemolytic transfusion reaction with hemoglobinuria and/or hemoglobinemia and no antibodies detectable by routine procedures; three of these patients had SCD.[31]

In 1980, Petz and Garratty[32] noted that the patient's own RBCs sometimes appeared to be destroyed during a DHTR. They described a case of SCD in which no new alloantibodies were detected at the time of the DHTR, but autoantibodies were detected in the serum and on the patient's RBCs. It was also noted that the direct antiglobulin test-negative RBCs from

TRANSFUSION 1997;37:357–361.

most patients with SCD have increased amounts of RBC-bound IgG; it was suggested that there may be an immune component of the hemolysis associated with SCD.[32-35] Other authors have described patients with SCD and alloantibodies who developed autoimmune hemolytic anemia.[36-38] Data from the series of DHTRs described above[13-26] show that, in 83 percent of the cases, the posttransfusion hemoglobin and hematocrit fell below the pretransfusion level; this suggests that the patient's own RBCs may have been undergoing increased destruction, without an autoimmune process being obvious. In this issue of **TRANSFUSION**, King et al.[25] and Petz et al.[26] address this phenomenon and come to rather different conclusions as to its cause.

King et al.[25] suggest that so-called "bystander hemolysis" may be a major mechanism in posttransfusion sickle cell crisis. The term "innocent bystander" was first used by Dameshek[39] to describe the mechanism by which immune complexes (e.g., drug–anti-drug complexes) could cause the lysis of innocent bystander cells (e.g., platelets and RBCs). Götze and Mueller-Eberhard[40] used the term "bystanding" to describe RBCs that became complement-sensitized (and sometimes lysed) by complement activation remote from the RBC. More recently, Petz used the term bystander hemolysis, in a broader sense, to describe phenomena in which one observes "immune hemolysis of cells that are negative for the antigen, against which the relevant antibody is directed."[41(p85)] King et al.[25] use a similar definition. As possible examples of bystander hemolysis, Petz[41] used paroxysmal nocturnal hemoglobinuria (PNH), in which HLA–anti-HLA complexes have been incriminated in the lysis of patients' RBCs during transfusion[42]; the hemolysis associated with drug-immune complexes; the hemolysis of transfused group O RBCs in minor ABO-incompatible bone marrow transplants, and, finally, the life-threatening hemolysis following transfusion in patients with SCD. He added a rider that "the interpretation of findings in SCD is complicated by the presence of chronic hemolytic anemia, by suppression of erythropoiesis by acute illness or transfusion, and by other factors. Thus, it has been difficult to conclude with certainty that the excessive drop in hematocrit after transfusion indicates destruction of the patient's own RBCs."[41(p91)]

In this edition of **TRANSFUSION**, King et al.[25] present five cases of sickle cell crisis associated with DHTRs following exchange transfusion. Three of the five patients had alloantibodies (anti-E, -S, -Jk^a, -Fy^a), identified after the exchange transfusion, that could have explained the DHTRs. One patient developed no new antibodies after transfusion, and one patient had no antibodies detected before or after exchange transfusion. Two patients received blood matched for antigens in the Rh, Kell, Kidd, Duffy, and MNSs systems; this did not prevent the DHTRs. The authors conclude that, in one patient, the loss of hemoglobin S after transfusion was probably due to suppression of endogenous erythropoiesis, but in one (possibly two) other patient(s), bystander hemolysis appeared to have been the cause. Unfortunately, no experimental data are presented to prove the hypothesis. The authors did not suggest what antigen–antibody reaction (in the absence of detectable RBC alloantibodies) caused complement activation and subsequent lysis of the patient's own RBCs. Nevertheless, I have a great deal of sympathy for their hypothesis.

In 1994, Test and Woolworth[43] showed a defect in regulation of the formation of the complement membrane attack complex (C5b-9) in sickle cells, particularly in the densest sickle cells.[43] The defect is characterized by increased binding of C5b-7 and C9 to the denser sickle cells, which results in the increased susceptibility of these RBCs to C5b-9-mediated (i.e., reactive) lysis. Thus, sickle cells appeared to be similar to PNH RBCs but the RBC defects were found to be different in the two conditions. In contrast to PNH RBCs, sickle cells were not quantitatively deficient in CD55 (decay-accelerating factor) or CD59 (membrane inhibitor of reactive lysis [MIRL]). It was suggested that, for reasons that are unknown at present, dysfunction of MIRL (CD59) is the most probable cause of abnormal C5b-9 formation in SCD.[43] Regardless of the reason, sickle cells are highly susceptible to reactive lysis[44] (a specific type of bystander lysis in which C5b-9 binds to cells and is independent of antibody, C1, C4, C2, and C3) and thus can be lysed by complement activation that occurs remote from the autologous sickle cells.

Although Test and Woolworth[43] did not discuss their findings in relation to transfusion reactions, I suggested recently that these findings provide a firm basis for the bystander lysis hypothesis.[44] I suggested that activation of complement could occur by the reaction of alloantibodies with transfused RBCs, which leads to the attachment of activated complement components to autologous RBCs; conversely, in the absence of RBC alloantibodies, other antibody reactions with transfused foreign antigens (e.g., HLA and plasma proteins) may cause complement activation.[44] Heal et al.[45] showed that >90 percent of patients given more than 20 units of platelets developed antibodies to a wide range of plasma proteins (e.g., albumin, fibrinogen, C2, C4). Thus, such antibodies may be present in many patients receiving multiple transfusions, which may lead to immune complex formation and possible complement activation during transfusion. It may be that the sickle cell defect described by Test and Woolworth[43] makes sickle cells (similar to PNH RBCs) much more susceptible to complement activation than are RBCs from other patients who receive transfusions. It is interesting to note that Salama and Mueller-Eckhardt[46] and Ness et al.[27] detected complement on the RBCs of most (100% and 60%, respectively) patients with DHTRs, even though the alloantibodies involved had specificities associated with antibodies (e.g., Rh system) that do not cause complement sensitization of RBCs in vitro. One may wonder if such RBC-bound complement is due to a bystander mechanism involving non-RBC antigen–antibody reactions. We may be seeing the same interactions as occur in SCD, but without the severe clinical effects associated with complement activa-

tion in SCD. This same effect could be occurring in post-transfusion purpura, a condition in which autologous platelets are destroyed and alloantibodies only are detectable.[44]

Another interesting aspect of complement activation in the transfused SCD patient is the relationship to the painful crises, which occurred during the DHTRs, in 91 percent of the cases described above.[13-19,25,26] The pathophysiology of the painful crises in SCD is poorly understood. One hypothesis is that the adherence of RBCs to activated endothelium initiates deoxygenation and sickling, which results in vaso-occlusion and necrotic tissue damage. Deoxygenation and sickling are also related to membrane phospholipid changes with the abnormal exposure of phosphatidylethanolamine and phosphatidylserine on the sickle cell membrane. Mold et al.[47] demonstrated that the membrane phospholipid changes that occur in sickle cells can initiate alternative complement pathway activation. In patients with intermittent pain, there was little evidence of complement activation at baseline, but increased levels of Bb and C3a were detected during painful crises; elevated levels of C3a and C4d were observed in patients with continuous pain. It is possible that this mechanism could lead to RBC-bound complement.

Also in this issue of **TRANSFUSION**, Petz et al.[26] describe five patients with SCD crises following transfusion. The histories were similar in many aspects to those described by King et al.,[25] but Petz et al.[26] came to different conclusions as to the cause. Using calculations of RBC production and destruction and incorporating correction factors for reticulocyte maturation, Petz et al.[26] suggest that the apparent increased rate of hemolysis of autologous RBCs was due to transfusion-induced suppression of erythropoiesis. They define a syndrome composed of a hemolytic transfusion reaction, pain crisis, marked reticulocytopenia, and more severe anemia after transfusion than was present before it. They emphasize 1) that the patients often have alloantibodies and may have autoantibodies, but some patients have no detectable antibodies; 2) that even RBCs that are phenotypically matched may be hemolyzed; 3) that withholding of further transfusions and steroid treatment appears to be the best therapy; and 4) that recovery is manifested by reticulocytosis. If one reviews the reticulocyte counts in reports of DHTRs in SCD,[6,7,9,14] marked reticulocytopenia at the time of DHTR is not an obvious feature; the reticulocyte counts range from 1.6 to 29 percent (mean, 11%; median, 8%), but these were not corrected absolute counts. It is important to note that Petz et al.[26] define marked reticulocytopenia in SCD as a significant decrease in *absolute* reticulocyte level from the patient's usual value.

It is difficult to argue with the calculations and conclusions reached by Petz et al.[26] King et al.[25] also suggested that one of their cases appeared to be due to suppression of erythropoiesis. It is well known that transfusion suppresses erythropoiesis,[48] and indeed, Squires et al.[49] suggested previously that this mechanism was the cause of severe anemia following a DHTR due to anti-Co^b in a patient with SCD. There are suggestions that, following a DHTR, the thalassemia patient's hemoglobin and hematocrit, like that of the SCD patient, sometimes drop below pretransfusion levels. Sirchia (Milan, Italy) has encountered this in 7 of 97 thalassemic patients (written communication, December, 1996). Giblett et al.[50] described a patient with thalassemia who had life-threatening anemia following a DHTR. It was suggested that this was due to temporary bone marrow suppression secondary to hypertransfusion. These findings could be used to support the hypothesis by Petz et al.,[26] although one cannot exclude bystander lysis, as no one has studied whether RBCs from thalassemics are as susceptible to reactive lysis as are those from patients with SCD.

Petz et al.[26] agree that suppression of erythropoiesis may not be the total answer, and they leave the door open for the bystander lysis mechanism to play a role. I find it of great interest that they and others[14,19,23] have found that steroid therapy is the best form of treatment. I know of no reason why steroids would affect transfusion-induced suppression of erythropoiesis; the fact that steroid therapy is the best form of treatment would suggest to me that an immune element (bystander mechanism?) is involved, at least in part.

Regardless of the mechanisms discussed, the articles by King et al.[25] and Petz et al.[26] serve to remind immunohematologists and physicians that a transfusion-induced life-threatening anemia plus other symptoms, especially acute pain, can occur after transfusion(s) in SCD patients. Although this was emphasized by Diamond et al.[14] as early as 1980, the "syndrome" is not mentioned in four of the best-known hematology textbooks that I reviewed for the purpose of preparing this editorial! Specific reviews on SCD mention alloimmunization and sometimes DHTRs (e.g., the excellent review by Wayne et al.[11] devotes 5 of its 20 columns to alloimmunization, but has only about three sentences on DHTRs), but I have found no such reviews that emphasize the SCD transfusion "syndrome" that is evaluated by King et al.[25] and Petz et al.[26]

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