Platelet Therapy and Red Cell Defect 
In Aplastic Anemia

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Transfusions, adrenal corticosteroids, androgens, and splenectomy in the treatment of aplastic anemia have yielded generally unsatisfactory results.\(^1\)\(^2\) Although hemorrhage due to thrombocytopenia is the most common cause of death in aplastic anemia, platelet transfusions have not been commonly used. The possibility that continued administration of platelets may result in immunization of the recipient and finally refractoriness to transfusion has limited the use of platelet transfusions. However, in studies of repeated transfusions of fresh platelets from the same donor to patients with acute leukemia, only rarely was progressive resistance to platelets noted.\(^3\)\(^4\)

The patient reported here received one to two platelet transfusions weekly for 14 months as prophylaxis and treatment of thrombocytopenia and hemorrhage, and the purpose of this report is to call attention to the efficacy of repeated platelet transfusions in this condition and to studies of the red cell life span during the recovery phase of his illness.

**Report of a Case**

An 8½-year-old boy was admitted to the National Cancer Institute on May 23, 1961. In February, 1961, he had tonsillitis for which he received 2 gm of chloramphenicol. In April, 1961, he developed fatigue, dyspnea on exertion, epistaxis, and purpura. The white blood cell count (WBC) was 1,400 per cubic millimeter, and the red cell count was \(2.5 \times 10^6\) per cubic millimeter. Specimen from a bone marrow biopsy was markedly hypoplastic; only plasma cells and lymphocytes were seen. The diagnosis of aplastic anemia was made, and he was started on methylprednisolone, 12 mg daily.

On the patient's admission to the National Cancer Institute on May 23, 1961, his chief complaint was dyspnea on exertion. Physical examination was unremarkable. The liver, spleen, and lymph glands were not palpable. Several ecchymotic areas were noted, but no petechiae were present. The hemoglobin (Hgb) was 5.1 gm per 100 ml and the WBC 3,500 per cubic millimeter with 18% neutrophils. The platelet count was 5,000 per cubic millimeter, and the reticulocytes were 0.4%.

The subsequent course and drug therapy are outlined in Fig 1. Six days after admission he had severe epistaxis and was given a transfusion of 1,225 ml of platelet-rich plasma \((4.8 \times 10^8\) platelets) which had been pooled from six different donors who contributed one unit each.* The platelet count rose from 2,000 to 110,000 per cubic millimeter one hour after transfusion and bleeding ceased immediately. As illustrated in Fig 1, ten days elapsed before the platelet count had fallen to less than 10,000 per cubic millimeter. On June 26, 1961, he developed bilateral flank pain and gross hematuria. Transfusion with 800 ml platelet-rich plasma \((3.2 \times 10^9\) platelets) from four different donors increased the platelet count from 2,000 to 73,000 per cubic millimeter one hour after the completion of the transfusion. Pooled platelet and packed red blood cells were his only form of treatment during this period except for fluoxymesterone.

In September, 1961, the patient developed jaundice which was considered to be a result of either androgen therapy or serum hepatitis. Androgens were discontinued, and prednisone was reinstituted (Fig 1). At the same time, the response to pooled platelet-rich plasma was noted to be progressively

\(^*\) A unit is the amount of platelets or platelet-rich plasma obtained from 500 ml whole blood.

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Fig 1.—Hematologic and transfusion data representing 14 months of observation of a patient with aplastic anemia. Vertical arrows under hemoglobin and platelet curves represent transfusion of red cells or platelets. The dose of red blood cells in units, and transfused platelets $\times 10^6$, is indicated by the height of the arrows. The actual calculated dose of platelets is shown by a solid line, and a dose estimated from the number of units transfused is shown by an interrupted line.
poorer. On Sept 20, 1961, he was transfused with two units of platelet concentrate (4.2 \times 10^{11} platelets) from an individual with chronic myelocytic leukemia (CML) whose platelet count varied from 1,000,000 to 3,000,000 per cubic millimeter. The patient’s platelet count rose from 5,000 to 50,000 per cubic millimeter. Through November, the response to pooled platelet-rich plasma from normal donors was lower than it had been initially (Fig 1). However, when platelets from the CML donor were transfused, the increment ranged from 50,000 to 132,000 per cubic millimeter. Furthermore, as this donor’s own platelet count became higher, the number of platelets obtainable from two units of plasma increased; thus extremely large amounts of platelets were transfused, and as the dose increased the post-transfusion increment increased as well.

In November and December, the number of granulocytes and reticulocytes in the peripheral blood rose to normal levels and reticulocytes began to appear, with values as high as 4.5% (Fig 1). His requirement for red blood cell transfusions decreased, and the hemoglobin fluctuated between 7.5 and 9.0 gm per 100 ml. The platelets always fell to very low levels (5,000 per cubic millimeter) if the patient was not transfused.

A schedule of plasmapheresis of the CML donor and transfusion of platelets every seven to ten days was maintained during the next six months. The very high doses of platelets noted in Fig 1 are from this single donor. Minor bleeding occurred only when the platelet count was lowest, and always ceased after transfusion of platelets.

Weekly injections of 300 mg of testosterone enanthate were instituted in January, 1962. By March, 1962, red blood cell transfusions were needed only rarely. A bone marrow aspiration in March showed an M:E ratio of 2:1 with 40% granulocytes, 40% lymphocytes, and 20% normoblasts. Cell indices in May showed macrocytosis, with mean corpuscular volume of 111 cu μ, mean corpuscular hemoglobin of 37μg, and mean corpuscular hemoglobin concentration of 34%. Hemoglobin electrophoresis was normal, but the alkali-resistant fraction was 5.5%. The glucose incubation test of Dacie* showed hemolysis of the patient’s red cells seven times greater than in glucose.

Red cell life span studies suggested that the spleen was contributing to the patient’s anemia. Therefore, on July 10, 1962, splenectomy was carried out. The patient was transfused with one unit of packed red blood cells and 13\times10^{11} platelets. The post-transfusion platelet increment was 68,000 per cubic millimeter, and the operation was performed without incident. The spleen was found to be slightly enlarged (160 gm). Microscopic examination showed a normal number of follicles, many of which had active germinal centers. The sinusoids were dilated and lined by prominent endothelial cells. On the second postoperative day, another transfusion was given resulting in a post-transfusion platelet count of 200,000 per cubic millimeter. Several days postoperatively the patient developed a renal calculus. After prophylactic transfusions with platelets to 470,000 per cubic millimeter, retrograde pyelography was done and no bleeding was encountered during the procedure.

After splenectomy the platelet count stabilized at approximately 15,000 per cubic millimeter for a month, and then gradually rose. No further transfusions were necessary, and at the time of the patient’s discharge in September of 1962, the platelet count was 35,000 per cubic millimeter, the hemoglobin 13.7 gm per 100 ml, the reticulocytes 0.6%, and the WBC 9,300 per cubic millimeter with 45% granulocytes (Fig 1). Endotoxin was given intravenously the month before splenectomy, and although the WBC and granulocyte counts were normal at this time, the normal granulocytosis which usually follows endotoxin injection was not seen. After splenectomy, although the WBC was the same, a normal increase was noted after endotoxin injection.

In February, 1963, a marrow aspiration was normocellular with rare megakaryocytes. Fifty per cent of the cells were normal maturing granulocytes, and 30% were erythroid precursors. Eleven per cent were mature lymphocytes, 5% plasma cells, 4% eosinophils. The WBC was 9,400 with 27% granulocytes and 67% lymphocytes. The reticulocytes were 1.2%, and the hemoglobin was 11.8 gm per 100 ml. Many Howell-Jolly bodies were observed in the red blood cells.

Red Blood Cell Survival Studies.—Splenectomy was considered in this patient because of the persistence of anemia in the presence of a regenerating bone marrow. In an attempt to predict the efficacy of splenectomy, red cell life span studies, using tritium- and phosphorous-32-labeled diisopropylfluorophosphate (DFP), were done in the patient, in an individual with an intact spleen, and in a splenectomized recipient.

The results of such studies prior to the patient’s splenectomy (Fig 2, upper left) showed moderate shortening of the patient’s red cell life span with nonlinear disappearance of the red cells, typical of random destruction. In contrast, normal donor cells in the patient had only a slightly shortened survival, and the disappearance pattern was characteristic of cells having the normal type of finite life span. The patient’s red cells were then given to a hematologically normal recipient with carcinoma of the breast (Fig 2, upper right), and in this environment a shortened red cell life span was demonstrated. Finally, red cell life span studies in a splenectomized patient with hemoglobin SC disease showed a shortened survival of the recipient’s own cells, and a normal survival of normal donor cells (Fig 2, lower left). For the first two weeks,
in this environment, the patient's cells disappeared at a rate slightly greater than normal, but showed the normal pattern of a finite life span. After this period of time, his red cells disappeared at an accelerated rate consistent with minor immunologic incompatibility and random destruction.11

Four months after splenectomy, the patient's red cell life span had increased almost to normal, and the pattern had changed from one of random destruction to the normal one of a finite life span (Fig 2, lower right). Presumably, even after splenectomy, the patient's intracorpuscular defect would be demonstrable if his labeled cells had been given to a patient with an intact spleen.

Comment

Hemorrhage is a direct or contributory cause of death in 56% to 92% of patients with aplastic anemia and pancytopenia.1-3 The incidence of remission, temporary or permanent, is variable, but occurs frequently enough (22% to 31%) to encourage intensive efforts directed toward supportive care and treatment. In one group of patients, the median time to remission was 19 months, while the median time to death for those who did not improve was seven months.4 Thus, it is reasonable to assume that the longer a patient with aplastic anemia is maintained by supportive measures, the more likely it is he will enter into a remission. When recovery does occur, it is usually first manifested by a decrease in the red blood cell transfusion requirement, followed by an increase in total leukocytes and absolute granulocytes. Platelets are usually the last formed element to return to safe levels.1 Thus, efforts directed at treatment and prevention of hemorrhage might well prevent the death of those patients who are closest to remission, that is, those in whom the return of erythrocyte and leukocyte levels to normal has already occurred.

Platelet transfusions have been used previously in the management of thrombocytopenia associated with aplastic anemia.3-5,12,13 However, they have been employed only when actual hemorrhage occurred and only to arrest manifest bleeding. Gaydos et al14 have shown that in patients with thrombocytopenia secondary to acute leukemia, the incidence of bleeding is inversely related to the platelet count, and that in patients with platelet counts of 10,000 per cubic millimeter

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Fig 2.—Results of red blood cell life span studies. Upper left, survival of autologous and normal cells in the patient (D); upper right, survival of patient's cells in a hematologically normal individual; lower left, survival of normal donor cells, patient's cells, and autologous cells in a splenectomized recipient with hemoglobin SC disease (B); lower right, after splenectomy: survival of autologous cells in the patient.
or below, there was some degree of hemorrhage on 50% to 90% of the days; and on 5% to 30% of the days, there was major, gross hemorrhage. The patient in this study was transfused with platelet-rich plasma or concentrate 75 times in an effort to prevent hemorrhage as well as to treat bleeding. His platelet count was maintained at a level of over 10,000 per cubic millimeter by platelet transfusion for 76% of the days that he was hospitalized. Of 412 days of observation before splenectomy, on only 93 days (22%) was his platelet count 10,000 per cubic millimeter or lower; bleeding was noted on 25 of these days, involving only the nose, gums, and skin. Since, after each platelet transfusion, his platelet count returned to low pretransfusion levels we might assume that if not transfused at all, then on all 412 days the platelet count would have been under 10,000 per cubic millimeter and the incidence of bleeding could have been many times greater.

Freireich et al. have shown that the post-transfusion increment of transfused platelets is directly related to the dose of platelets transfused. This relationship is illustrated for this case in Fig. 1. A donor with thrombocytosis (CML) was found whose platelets produced excellent post-transfusion platelet increments and survival. Platelets from this donor were transfused a total of 31 times over nine months without evidence of diminished response in the recipient. Furthermore, in January and February platelet transfusions from two normal donors were given several times, and although the doses were comparatively small the responses were satisfactory for the number of platelets transfused. This illustrates that the patient was responsive to platelets from donors other than the person with thrombocytosis, making it likely that the continued excellent response to this one donor was probably not due to any qualitative difference in his platelets but merely that there were more of them. Androgens and adrenal corticosteroids were employed as recommended by Shahidi and Diamond, but it is impossible in any individual patient to determine whether improvement was related to their administration or not.

Shortened red cell life span is not uncommon in aplastic anemia and has been reported several times. However, red cell life span studies in this patient demonstrated an intracorpuscular red blood cell defect and suggested the role played by the spleen in shortening the survival of these defective cells. Macrocystic cells, such as were noted in this patient, are commonly seen in patients with aplastic anemia. This finding supports the concept of an intracorpuscular defect. Macrocystosis of this type may be a typical marrow response to toxins and hemorrhage. In the acutely bled rabbit, rat, and dog it has been shown that the red cells produced in the first few days after hemorrhage have a shortened life span. It is interesting to speculate that the situation in this patient was comparable to that of the acutely bled animals where maximal stimulation of the marrow results in defective red cell synthesis. Shahidi et al. offer additional evidence for an intracorpuscular defect with the discovery of increased amounts of alkali-resistant hemoglobin in these patients. Our patient has hemoglobin F of values of over 5% before, and nine months after, splenectomy.

Splenectomy has been performed in many instances in patients with aplastic anemia. Most cases in which remission occurred after splenectomy were in patients with demonstrable hemolysis, or with increasing marrow cellularity over an extended period of observation. Those who were splenectomized may have had a better chance of remission because they had had their disease a longer time prior to surgery. Nevertheless, dramatic remissions may occur after splenectomy. The role of the spleen as a factor in this patient’s anemia was documented preoperatively by the transfusion of red cells into a normal and a splenectomized patient, and also in the patient himself four months after splenectomy.
BLEEDING: ANEMIA

Bleeding has not occurred since the splenectomy, and although the patient has not yet made a complete recovery there has been marked improvement, the platelet count has remained at about 60,000 per cubic millimeter, and he has been able to resume normal activity at home.

SUMMARY

The management of aplastic anemia in an 8-year-old boy is discussed, with emphasis on the usefulness of repeated platelet transfusions as prophylaxis for thrombocytopenia and hemorrhage. In addition, red cell life span studies prior to splenectomy demonstrated a shortened red cell survival which was corrected by splenectomy. Evidence for intrinsic abnormality of the patient's red blood cells is presented. Thrombocytopenia was favorably influenced by splenectomy as well.

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GENERIC AND TRADE NAMES OF DRUGS

Methylprednisolone—Medrol.
Chloramphenicol—Chloromycetin.
Fluoxymesterone—Halotestin, Ulltandren.
Prednisone—Deltasone, Delta, Meticorten, Para-cort.
Testosterone enanthate—Delastestryl.

REFERENCES


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