PLATELET TRANSFUSIONS
Recent Progress and Controversial Issues

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The transfusion of platelet concentrates to arrest hemorrhage in patients with thrombocytopenia became a common therapeutic modality in the early 1960s when plastic bags were made available for blood collection and separation of blood components. Platelet replacement therapy has expanded enormously during the past decade. The preparation of platelet concentrates by the American Red Cross has increased from 79,000 units in 1969 to 286,000 units in 1972, to 1,556,328 units in the year 1978–79 and 1,702,036 in 1979–80.1 This American agency is presumed to supply only about one half of the blood and blood components used in the United States. In great part, this rapid growth has been associated with protocol treatment programs for acute leukemia. Much has been learned from these observations, although important questions still remain unanswered and are presently debated.

Our present review will be limited to a critical evaluation of a few still controversial aspects of platelet transfusion therapy including: 1) Indications for platelet transfusions. 2) Quality controls for platelet preparations. 3) The short term storage of platelets. 4) Preservation of platelets by freezing with DMSO. 5) The problem of platelet alloimmunization.

Indications for Platelet Transfusions

It is generally accepted that patients with thrombocytopenia secondary to failure of platelet production by the bone marrow are the best candidates for platelet transfusion therapy because the in vivo recovery and survival of transfused platelets is usually close to normal in these patients. It is also accepted that if platelet transfusions are needed in patients with thrombocytopenia secondary to "hypersplenism", that is to pooling of the platelets in an enlarged spleen,
more platelets will be needed to obtain a sufficient platelet increment. The debated question still remains, however, as to whether platelet transfusions are at all beneficial in patients with thrombocytopenia secondary to increased platelet destruction due to antiplatelet antibody (auto-antibody, drug-dependent antibody), or in patients with thrombocytopenia due to marrow failure who, however, have been alloimmunized to donor platelets by previous transfusions. Several workers\(^2\) \(^3\) have maintained that platelet transfusions should not be given to these patients even in the case of important hemorrhage. We differ on this issue on the basis of the experimental results of Nagasawa et al.\(^4\) In rabbits alloimmunized to platelets, survival of \(^5\)Cr labeled platelets from donor rabbits became extremely reduced. However, donor platelet survival measured after a large infusion of \(4 \times 10^{10}\) freshly prepared platelets was nearly normal during the first 24 hours. In vitro tests proved that the large infusion of allogenic platelets had, by absorption, caused a significant reduction of the serum alloantibody. Non-viable and, even, lyophilized platelets could produce this effect, although to a lesser extent (Fig. 1). A similar result could be demonstrated also in patients with thrombocytopenia due to bone marrow failure, but alloimmunized to platelets by prior transfusions\(^4\). In conclusion, in the presence of antiplatelet antibody in the circulation, only large numbers of platelets will produce an effective result on hemostasis.

There is no precise threshold platelet level below which severe hemorrhage will undoubtedly occur although hemorrhage is common with platelet below 5,000 to 10,000/cu mm. It is, therefore, difficult to justify the concept of prophylactic platelet transfusions particularly in face of the fact that indiscriminate
transfusion of platelets most commonly leads to inability to control bleeding because of alloimmunization. Two recent studies\(^5\)\(^6\) on patients with acute leukemia clearly indicate that there is no documented evidence to the fact that prophylactic platelet transfusions can reduce the incidence of bleeding deaths, number of red cell transfusions, incidence of remission and length of survival. We prefer, therefore, to discourage this practice accepting, however, reasonable exceptions for patients in whom there are clear signs of imminent, overt bleeding related to the platelet deficiency.

Our current recommendations for platelet transfusions are the following: 1) To transfuse patients with severe hemorrhage or signs of impending hemorrhage, (persistent headache, eye hemorrhages, hematuria, melena, hematemesis, rapidly increasing purpura while platelet counts are less than 10,000/mm\(^3\), and in preparation for surgery). 2) To use 5—10 units per transfusion. 3) Immunologic thrombocytopenia is no valid reason for avoiding platelet transfusions. 4) In case of immunologic thrombocytopenia or alloimmunization, to transfuse 20—30 units at once to adsorb antibody. 5) Old platelets or lyophilized platelets can also be used for antibody absorption, after which 5—10 units of fresh platelets must be infused.

**Quality Controls for Platelet Preparations**

Measuring the clinical effectiveness of transfused platelets in bleeding patients is a very difficult task because there are many factors which can influence the result of platelet transfusion in a given patient independently of the good quality of the platelet preparation used. These difficulties, however, do not justify the present complacency of clinicians towards blood banks and blood centers. We must admit that quality controls for platelet preparations are, at present, grossly inadequate. Most blood centers limit themselves to examine their platelet concentrates by platelet counting performed at regular or irregular intervals. Our recent spot-check on 60 random platelet concentrates from a non-specified Blood Center in the United States, gave a mean value of \(3.39 \times 10^{10}\) platelets per unit with a S.D. of \(1.43 \times 10^{10}\) and a range varying from 0.8 to \(7.1 \times 10^{10}\). Usually, the lowest acceptable limit for platelets collected from a unit of 450 ml of blood is \(5.5 \times 10^{10}\). Only 8 % of the platelet concentrates examined were above that limit.

In the future, in addition to the number, the quality of the platelets to be transfused will have to be examined and, also, their hemostatic effect on the bleeding patient. There are a variety of tests which could lend themselves to an effective system of quality control for platelet preparations. One group of tests to be performed on the patient could include one or more of the following: 1) increment in platelet count, 2) platelet survival by labeling with \(^{51}\)Cr, and
3) post-infusion bleeding time by template. The second category to be performed on the platelet concentrates could include: 1) platelet number, 2) platelet aggregation with ADP, collagen, epinephrine, thrombin, ristocetin, 3) in vitro uptake or release of $^{14}$C-labeled serotonin, 4) Ca$^{++}$ uptake or release, 5) platelet content of ATP, ADP, AMP, 6) platelet factor 3, 7) platelet factor 4, and 8) the in vitro platelet response to hypotonic shock (platelet reversal reaction). Which combination of tests will be the most practical and accurate in assuring efficacy of platelet preparations is still unknown. The platelet reversal reaction appears to be the most promising in vitro test for preserved platelets (Fig. 2). Large scale studies on this subject are warranted.

The Short Term Storage of Platelets

It was first realized in 1960 that human platelets stored at 4°C partially lost viability within less than three hours, and that after 24 hours of storage at this temperature, most of the platelets were non-viable and, therefore, not effective.
on hemostasis. Compared with fresh platelets, less than 50 per cent of the stored platelets would reappear in the circulation after infusion, and survived only 2.5 rather than the normal 8.5 days. These findings focused attention on the problem of platelet preservation. Since then, progress has been slow, but significant. The skyrocketing demand for platelet transfusions in the treatment of patients with thrombocytopenic hemorrhage, has exerted a sense of urgency on this complex field.

In this area of research three milestones deserve special mention 1) The introduction by Aster and Jandl, in 1964, of ACD as the anticoagulant of choice for platelet collection and separation. This greatly improved the in vivo recovery value of platelets after infusion and, to a smaller extent, the recovery value and the life span of platelets stored for short intervals at 4°C. It was seen that the mean recovery value for ACD platelets stored for 24 hours, was nearly one half of the mean value for fresh platelets, while with stored EDTA platelets only one third (35%) of the respective fresh platelet value was obtained. 2) The finding by Murphy and Gardner, in 1969, that viability of platelets during storage is better maintained at 22°C rather than at 4°C represents the second milestone. Fig. 3 reports our experience in this field of study. After 48 hours of storage at 22°C, platelets had an almost insignificant reduction in survival. The rather dramatic difference in survival values of 22°C platelets clearly indicated that platelet viability for transfusion purposes is best maintained at 22°C rather than at 4°C. 3) The third milestone is represented by the finding of a successful method of platelet preservation by freezing using dimethylsulfoxide (DMSO) as a cryoprotective agent. By this method, platelets

![Fig. 3 After 48 hours of storage at 22°C, platelets had a very moderate reduction in survival. Survival was severely reduced at 4°C. Autologous platelets were used in these experiments.](image-url)
can be preserved viable for indefinite periods of time.\textsuperscript{13–15)}

Storage of platelets at 22°C currently represents the easiest and most widely used method of preserving viable platelets for short intervals. However, at 22°C the platelets appear to partially lose several functions. In our experiments, after 24 hours of storage, platelet aggregation with ADP was only one third of the value obtained before storage. Collagen induced aggregation was two thirds of that value. However, recirculation appears to lead to recuperation of this function and after infusion in thrombocytopenic patients, hemostasis becomes corrected.\textsuperscript{16)} A number of factors seem to contribute to the observed deleterious effect of room temperature storage on platelet aggregability. These include\textsuperscript{2)} changes in pH of the medium, loss of platelet glycogen, and also membrane alterations as demonstrated by a reduction of the platelet reaction to osmotic shock (the so-called reversal reaction)\textsuperscript{7)} \textsuperscript{8)} and by a decreased platelet uptake of Ca\textsuperscript{2+}.

The problem encountered with liquid storage of platelets at 4°C was first identified by Zucker and Borrelli in 1954,\textsuperscript{17)} when they observed a morphological change from disc to sphere of the platelets at this temperature. White and Krivit,\textsuperscript{18)} using electronmicroscopy, observed microtubular bundle dissociation with complete disappearance of platelet microtubuli. Simultaneously, there was an increase in microfilaments, the characteristics and properties of which are still obscure. Ikeda and Steiner,\textsuperscript{19)} in our laboratory, separated microtubule protein in pure form. By subjecting this protein to 4°C temperature, they have observed depolymerization which is reversible only when exposure to cold is limited to less than one hour. The reduced electrophoretic mobility and the reduced in vitro reaction to osmotic shock also observed after chilling the platelets, indicate important alterations in membrane functions.\textsuperscript{7)} Furthermore, spontaneous aggregation upon rewarming is well known to occur in chilled platelets. Undoubtedly, storage at 4°C preserves the functional qualities of the platelets as Becker et al. have demonstrated,\textsuperscript{20)} but because of their severely limited ability to circulate, hemostatic effectiveness is reduced to a brief period following infusion. Platelet viability largely relates to hemostatic effectiveness in the field of platelet storage and certainly is the first prerequisite for the platelets to be effective on hemostasis. Platelets stored at 4°C even for only 24 hrs would be cost ineffective mostly because of their reduced survival.

The T 1/2 values of the survival curves obtained by Murphy et al. after storage of the platelet concentrates for 24 hours at different temperatures around 22°C, when plotted, showed a very steep course below 22°C.\textsuperscript{12)} This indicates the importance of maintaining a precisely constant storage temperature during the usual period of 24 to 72 hours presently accepted by the American Association of Blood Banks. A drop in temperature of 2°C would considerably reduce the
T1/2 value of the preserved platelets.

While most blood centers and blood banks are presently processing and storing platelets for up to 72 hours at 22°C, it should be realized that after 72 hrs at this temperature platelets have a mean, in vivo recovery value which is always less than one half the value obtained with fresh platelets and survival is usually less than 6 days. Furthermore, the platelet survival curves obtained after 72 hrs of storage are mostly exponential. Viability of these platelets is, therefore, drastically reduced to such values that one wonders whether arrest of hemorrhage can be obtained with these platelets and how many units should be infused to obtain a significant result in a bleeding patient. Furthermore, if the platelet concentrate contains too many white cells or too little plasma (<50 ml), the pH may drop to <6 during the 72 hrs. In this case, the platelets would have no viability after infusion and, therefore, no effect on hemostasis. Platelet function is also drastically reduced after this period of storage. All considered, we strongly discourage the use of platelets stored at 22°C for as long as 72 hrs unless each unit is accurately examined and tested before infusion.

It is obvious that no matter what method or temperature is used to store platelets, injuries are sustained by the cells which reduce their viability and/or their function. Underlying causes and mechanisms operative in the production of these preservation injuries are largely unknown. Corrective actions have so far been empirical and have lead to only partial success. Presently, research on platelet preservation has reached an impasse probably because all the empirical "strikes" have been exhausted. Now, therefore, we must go back to the work-bench. We believe that a deeper understanding of the basic biochemical and biophysical mechanisms which assure cellular integrity and functional competence of fresh and stored platelets is essential for any further progress in this field. With regard to platelet preservation, membrane structure, organization and function constitute an important area of platelet research in which our present knowledge must be considered rudimentary. This field appears to us of greatest importance for cell preservation in general and platelet storage in particular.

Platelet Storage by Freezing with DMSO

Freezing platelets with dimethylsulfoxide (DMSO) has become feasible during the past eight years yielding platelet preparations which, compared with fresh platelets, display only a moderately decreased aggregation. This defect, however, appears to rapidly improve upon infusion in vivo. In our experiments, the radioactive 51Cr label was added to the platelets before freezing and viability of the platelets was measured by autologous survival using the 51Cr label. The peak recovery value of platelet radioactivity in the circulation was on the average 63 % (normal, 66 %). The survival time was normal or nearly so in all experi-
ments.1)

This new, successful platelet freezing method is based on a few technical principles which, in our studies, have appeared essential: 1) the use of plasma as the platelet suspending medium; 2) a final 5% concentration of DMSO; 3) the very gradual addition and removal of DMSO to minimize the osmotic shock to the platelets; 4) slow cooling at −1° to −2°C per min and rapid thawing in less than one min; 5) the use of protected plastic bags which can maintain the platelet concentrate in a relatively thin film. Human platelets frozen by this method and preserved at −79°C for one to four days or at −85 for 9 to 15 months maintained a nearly normal in vivo survival capacity after labeling with 51Cr, and an excellent hemostatic effectiveness. In Fig. 4, 14 experiments are reported by the infusion of frozen platelets in severely thrombocytopenic patients. Hemostatic effectiveness of the frozen platelets was equated with the capacity of the platelets to shorten the prolonged bleeding time in the patients. The 14 thrombocytopenic patients studied all had severe bone marrow depression due either to idiopathic aplastic anemia or to chemotherapy for acute leukemia. Eleven patients were adults and three were children. The platelet counts in the patients were 16,000—40,000 per mm³ before infusion of the frozen platelets. The bleeding time was measured by the template method as described by Mielke et al. The normal value by this method is 4—8 min in our laboratory. In all patients, the bleeding time was severely prolonged, 19 min in one patient, 20 min in a second patient, longer than 25 min in two
patients, and longer than 30 min in the others. No bleeding time was measured beyond 30 min. The platelet counts were also measured before and after infusion of the frozen platelets. Four units of frozen platelets were infused in each of the adult patients and two units in each of the children. One platelet unit contained an average number of $63 \times 10^9$ platelets ($46-76 \times 10^9$). Fifteen minutes after infusion of the frozen platelets, there was a marked reduction in the bleeding time to an average value of 14 min. The bleeding time remained beyond 30 min in only two patients. In all patients, however, the bleeding time became shorter 3 hr post-infusion with an average value of 10 min closely approaching the normal range of 4—8 min. After 24 hr from infusion, the bleeding time became prolonged with a mean value of 19 min in 8 patients while it was beyond 25 min in 1 patient and beyond 30 min in 2 patients.

Platelet increments after infusion of the frozen platelets are reported in the lower portion of Fig. 4. Values are expressed as increments of platelet number per mm$^3$ and proportioned to the infusion of $10^{11}$ platelets per m$^2$ of body surface. Before infusion, the platelet counts varied between 16,000 and 40,000 per mm$^3$ with a mean value of 23,000 per mm$^3$. The mean platelet increment measured 15 min after infusion was 14,700 (11,800—22,000) per mm$^3$ and was on most occasions, similar to what is observed with the infusion of freshly prepared platelet concentrates (left column). The increment values obtained soon after infusion were the highest and were decreased 3 hr and, even more, 24 hr post-infusion. Shortening of the bleeding time in the thrombocytopenic patients soon after platelet infusion was of a promptness and degree almost similar to that usually obtained with freshly prepared platelets. The effect of the infused platelets on hemostasis was more pronounced 3 hr than 15 min after infusion, while increments in platelet count were highest soon after infusion. This discrepancy between increase in platelet count and effect on shortening of the bleeding time is not seen with fresh platelets and indicates to us that the frozen platelets carried a functional lesion which temporarily impaired their hemostatic effectiveness without shortening their survival. Three hours post-infusion, increments in platelet count were lower, but shortening of bleeding time was more pronounced, clearly indicating that the platelets were recovering from the storage lesion. Analysis of the individual results showed no difference between platelets stored for 1 day and those stored for 2—4 days at $-79^\circ$C, and those stored for 9 to 15 months at $-85^\circ$C (three cases represented in Fig. 4 by squares), indicating that freezing and thawing per se rather than length of storage, were responsible for the platelet damage. After thawing, however, the previously frozen platelets could not withstand storage without loss of viability for more than 6 hours. Their shelf life at room temperature was, therefore, very limited.
It was later emphasized that the time-consuming rule of gradually lowering the cooling temperature at the rate of 1°C per min in a special slow-freezing chamber can be somewhat relaxed. Platelet concentrates placed on a shelf of a laboratory freezer (−2° to −3° per min) proved to be equally well preserved.24) Other rules for platelet freezing must, however, be strictly preserved. When we prepared multiple platelet concentrates pooled in one single polyolefin bag with a change in volume from 30 to 60 ml of the platelet concentrate, thickness of the freezing pack increased causing (as any cryobiologist would have probably predicted) a gross damage to the platelets. The in vivo survival curves of these platelets were shorter and had lower recovery values than in the curves obtained when single 30 ml units were frozen.

The reason that the DMSO method of platelet freezing has not yet been routinely applied in hospitals is the lack of approval for prescriptive use by the Food and Drug Administration. Although the DMSO-containing medium is removed, each platelet concentrate (derived from 500 ml blood) still contains 17—45 mg of this compound at the time of infusion in the patient. To reduce the amount of DMSO to minimal traces, we applied two washings and used 150 ml rather than regular (300 ml) bags. The method became, however, cumbersome and a further, small loss of platelets was recorded. There is no evidence that DMSO in such small amounts can cause any side effects in human beings, but there is apparently not enough volume of documentation as yet to allow the F.D.A. to approve it for prescriptive use. The procedure, therefore, although widely utilized by investigators in clinical cases, is still entirely experimental.

Frozen platelets will most probably be made available to our hospitals in the future. We believe, however, that frozen platelets will never replace the use of fresh and liquid stored platelets because of practicality and cost. A few indications of frozen platelets seem important to us: 1) Platelets could be harvested and stored for long intervals as a byproduct of blood banking. Presently, about
25 percent of the harvested platelets are wasted after 72 hrs of storage at 22°C. 2) HL-A typed and matched platelets could be harvested and preserved for long intervals for use in compatible recipients. 3) Autologous platelet transfusions could also be utilized by freezing the patient's own platelets collected by cell separator prior to the occurrence of thrombocytopenia, e.g. in patients scheduled to receive chemotherapy for a malignant process (Fig. 5). Finally, 4) A national or international pool of frozen platelets could be organized and stored indefinitely for use in special emergencies, as in case of an atomic disaster.

The Problem of Platelet Alloimmunization

It has long been known that blood platelets are alloantigenic and that repeated blood or platelet transfusions may cause unresponsiveness to subsequent infusions of donor platelets. When this refractory state has been reached, no increment in platelet count follows the platelet infusion. Refractoriness to platelets is a serious clinical problem in severely thrombocytopenic patients and has stimulated studies for a number of years. In the early 1960s, the technique of 51Cr labeling for the measurement of allogenic platelet survival in sensitized recipients

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**NON-IDENTICAL TWINS WITH APPARENTLY "IDENTICAL" RED BLOOD CELLS**

*Fig. 6* A pair of non-identical twins with "identical" red cell antigens. Cross-skin grafts were rapidly rejected. After 6 infusions of platelets from the donor twin, the recipient brother showed marked reduction in survival of the infused fraternal platelets. An infusion of "nonspecific" platelets from an unrelated donor showed an almost normal survival.
offered a new approach to the study of platelet alloimmunization in human sub-
jects and provided the first highly significant group of results which can be
summarized as follows: 27) 1) Alloimmunization in response to regularly spaced
platelet infusions, developed in all volunteer recipients after 2 to 8 platelet infu-
sions. Survival of allogenic platelets became drastically reduced. 2) No differ-
ence in time and severity of alloimmunization was observed whether the platelets
infused were from ABO-compatible or incompatible donors. 3) No significant
difference in time and severity of alloimmunization could be observed whether
the volunteers were infused with platelets from one single donor or from several
donors. 4) Alloimmunization resulting from exposure to platelets from one
single donor was also effective against platelets from other donors although
“specific” platelets, i.e. platelets from the immunizing donor, often had a shorter
life span than “non-specific” platelets from a new, random donor. 5) Thrombo-
cytopenia in the alloimmunized recipients never occurred.

These findings suggested that platelets have a complex antigenic structure with
a large variety of antigens being expressed on their surface so that a recipient
would probably always lack one or more of the antigens present in the platelets
of any donor.

Our studies on a pair of non-identical male twins with “identical” red cell
antigens (28 could be tested), provided the most conclusive information. Their
non-identity was confirmed by the rapid rejection of skin crossgrafts. After 6
infusions of platelets from the donor twin, the recipient brother showed marked
reduction in viability of the infused “fraternal” platelets. A subsequent infusion
of platelets from another donor showed an almost normal viability (Fig. 6).
This experiment confirmed the independence of platelet antigens from red cell
antigens. Furthermore, a detailed study of the platelet viability curve obtained
in the recipient twin with the first infusion of platelets from the brother showed
a significant shortening of the life span of the allogenic platelets. Since the re-
cipient had never received blood or platelet transfusions, the possibility was
raised that the skin allograft had sensitized the recipient twin against the fraternal
platelets, and the concept was developed that blood platelets may contain anti-
gens shared by other body tissues. This problem was evaluated by the use of
a rabbit model.28) After three skin allografts, viability of platelets from the
“specific” skin donor was reduced in all experiments (Fig. 7). Also, a direct
relationship between number of skin-allografts and degree of reduction in the
life span of allogenic platelets could be shown. In subsequent experiments,29)
rabbits were first sensitized by one intradermal injection of allogenic platelets.
The rejection time of subsequently implanted skin allografts was, then, deter-
mined. In 42 per cent of the platelet-sensitized animals the rejection time of
“specific” allografts, that is allografts from the respective platelet donors, was
significantly shortened, i.e. below the limit of 2 S.D. from the mean value observed in non-immunized rabbits. "Non-specific" allografts, i.e. allografts obtained from animals other than the platelet donors, were also rejected sooner than normal, but in only 10 per cent of the animals. When rabbits were sensitized by one skin allograft, the rejection time of the second allograft was, as expected, shorter than in practically all experiments.

Two results were particularly relevant: that after alloimmunization with "pure" platelets, a significant number of animals displayed a second-set type of rejection of allogenic skin and that the rejection was more evident for "specific" than for "non-specific" grafts. It was concluded that: 1) Blood platelets and skin have antigens in common. 2) These antigens are not completely individual specific 3) Blood platelets contain histocompatibility antigens. On this evidence, we predicted in 1961, that reduced viability of allogenic platelets and, therefore, reduced effect on hemostasis would potentially be an inevitable event in patients receiving repeated blood or platelet transfusions.

A number of group specific platelet alloantigens have been described since 1957, when Moulinier defined antigen "Duzo" using an exceptional antibody from a mother whose infant had neonatal purpura. These antigens, the "Zw",...
“Ko”, PL$^{A_1}$, PL$^{A_2}$, and others, have the characteristic of being shared by a high percentage of the human population and only account for rare occurrences of platelet alloimmunization, mostly feto-maternal.\textsuperscript{30}

Based on the definition of the HL-A histocompatibility system and lymphocytotoxicity testing to which Terasaki,\textsuperscript{32} among others, had greatly contributed, R. Yankee and associates\textsuperscript{33} in 1969, observed excellent responses to the infusion of platelets from HLA-identical siblings given to multitransfused patients who had become refractory to platelets from both unrelated random donors and non-HLA matched family members. It became increasingly evident that long-term platelet transfusion support would cause progressive refractoriness to platelets obtained from random donors and that in these patients, transfusions of platelets from HLA-compatible family members would still be effective. In 1972, Thorsby\textsuperscript{35} and associates demonstrated the following: 1) an unrelated, HLA-compatible donor could be successfully utilized to prolong platelet support for an alloimmunized, thrombocytopenic recipient, 2) the number of available donors could be increased by transfusing platelets from persons whose HLA match with a given recipient was of grade B, i.e. all antigens of the donor were present in the recipient, but one (B$_1$) or more (B$_2$) of the recipient’s antigens were not present in the donor, 3) the use of platelets from a single random donor was preferable to the use of platelets from multiple random donors because by using the former, occurrence of alloimmunization was delayed.

These results led to the conclusion that the HLA genetic locus as determined by the HLA antigen profile on lymphocytes (J. Dausset et al. 1967),\textsuperscript{36} plays a primary role in the development of platelet alloimmunization, and that the same genetic locus that controls the inheritance of HLA antigens is the major determinant in the expression of allograft immunogenicity and, simultaneously, of platelet alloimmunization. There was the possibility that platelet alloimmunization could be totally prevented by the use of HLA compatible donors. Unfortunately, the HLA system is one of the most highly polymorphic of the genetic systems of man, and more than 40 antigens have now been defined at loci A and B alone. Thus, unless an HLA matched sibling can be found, the problem of locating compatible donors is formidable. Opelz, Mickey and Terasaki\textsuperscript{37} have calculated that more than 50,000 HLA typed donors are necessary to assure a 50 per cent probability of finding 10 perfectly matched donors for each of a pool of 100 potential recipients. This would almost be an impossible and certainly a very expensive project. Continued investigation has now revealed that, despite the use of HLA phenotypically matched donors, not all immunized recipients achieve compatible post-transfusion platelet increments.\textsuperscript{38} Administration of leukocyte-poor platelets may be of partial help in producing effective post-transfusion platelet increments. Also, better results are obtained by using donors of
match grade A and B₁ rather than of grade B₂ and C. But these studies have shown, and further experience has confirmed, that with continued transfusion exposure, loss of effectiveness of platelet concentrates is still obtained, regardless of the HLA match grade.³) Conversely, failure of recipients to produce antibodies directed towards HLA antigen specificities present on donor cells and not present in the recipient has also been observed.³) Proven non-responders, however, are rare, particularly after a protracted, regularly spaced exposure to large numbers of allogenic platelets. Our clinical experience indicates that they are not 30 per cent of all recipients as may be true in the dog.³) In our experiments, dog platelets were less alloimmunogenic than human platelets.

While further studies on basic concepts and on the structure of the HLA system are being done and the field of less immunogenic cross-reactive antigens⁹) ⁴⁰) resulting in increased tolerance to platelets is being elucidated, we are faced with the problem of an increasing number of bleeding, thrombocytopenic patients who have become refractory to platelet transfusions. In our experience, splenectomy as well as immunosuppressive therapy have been of little or no help in severely alloimmunized recipients.⁴¹) Recent research, however, has provided significant data with regard to these difficult clinical situations. The following deserves mention: 1) a new method for the determination of platelet associated immunoglobulin G has been developed by Dr. Sugiura et al.⁴²) ⁴³) which is based on spectrofluorophotometry and uses fluorescein isothiocyanate as a marker of IgG from antihuman IgG goat serum. Measurement of platelet-associated IgG by this method is rapid, quantitative, reproducible, sensitive and inexpensive. All patients with immune thrombocytopenia (ITP) had levels of PA-IgG increased above the mean plus 2 S.D. when the platelet count was below 100,000/ mm³. Using the indirect assay, serum levels of PA-IgG were found increased in 86 per cent of these patients. The PA-IgG decreased progressively with treatment and improvement of the platelet count in the patients. Dr. Sugiura also indicated that this method, in its indirect variety, could be used to measure the presence of alloantibody reacting with donor platelets.⁴³) More recently, this test was adapted by T.J. Myers et al.⁴⁴) ⁴⁵) in our laboratory, to the detection of platelet alloimmunity in multitransfused patients, and to the selection of prospective platelet donors for alloimmunized recipients. Survival of ⁵¹Cr-labeled donor platelets in the recipients served as the control. Sera from 8 of 12 multitransfused patients reacted with a panel of random donor platelets as shown by increased PA-IgG. Absorption studies showed that the PA-IgG reacted as an alloantibody. Sera from 4 patients produced no increase of PA-IgG on random platelets incubated with the sera. On all occasions, the PA-IgG assay predicted the survival time of donor platelets in the multitransfused recipients as measured by ⁵¹Cr. The T 1/2 was less than 30 min with all donor platelets presumed
incompatible, while it was normal or nearly so with platelets presumed compatible. A strong inverse correlation was observed between PA-IgG and platelet survival values. Platelet aggregometry and serotonin release studies simultaneously done, identified only few of the alloimmunized cases, and showed poor correlation with amounts of PA-IgG. Platelet aggregometry identified only 8 of the 17 survival results, serotonin release identified 7, and lymphocytotoxicity identified 10 of the 17 patients. We believe that a simple and reliable method has now been obtained which may be of particular value in detecting alloimmunization to platelets, and in screening platelet donors for alloimmunized patients, even when HLA testing may show a mismatch between donor and recipient.

In conclusion: 1) Platelet alloimmunization with refractoriness to platelet transfusions is inevitable with continued exposure to allogenic platelets, except in rate unpredictable cases of non-responders. 2) Presence of alloimmunization to platelets can best be demonstrated by the in vitro PA-IgG quantitative assay described by K. Sugiura et al.42) 43) 3) Cross-matching for the selection of compatible donors for alloimmunized recipients is presently best done using the indirect PA-IgG assay as T. Myers et al. have demonstrated.44) 45) 4) Continued transfusion of platelets from a single random donor is preferable to the transfusion of platelets from multiple random donors because recipients alloimmunized to platelets from one donor, can often receive temporary support from the transfusion of a new random donor.

The latter point has recently been debated on the basis of a few experiments in dogs.3) We maintain that there is solid documentation in the literature for the opposite view on this subject from experiments in animals and human subjects.35) 28) In our experience, platelet alloimmunization in the dog is less frequent than in man, and a few other species differences46) have also been observed in the past.

On the basis of our present knowledge, we believe that: 1) HLA-compatible platelets should first be used in patients in need of protracted platelet transfusion therapy. 2) The rise of alloimmunization should be monitored by the use of the quantitative PA-IgG indirect assay.43) 3) Selection of compatible related or unrelated donors should be done by the PA-IgG compatibility matching test.44) 4) Platelet pheresis of compatible donors associated with platelet preservation by freezing should provide adequate continued platelet support for chronic, severely thrombocytopenic patients. Although expensive, the program is feasible.

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