An Animal Model of Allogeneic Donor Platelet Refractoriness: The Effect of the Time of Leukodepletion

By Morris A. Blajchman, Leslie Bardossy, Raleigh A. Carmen, Mindy Goldman, Nancy M. Heddle, and Dharam P. Singal

Approximately 50% of multi-transfused individuals become refractory to random donor platelets. Recent clinical data suggest that those patients receiving leukocyte-depleted blood products are less likely to become refractory to random donor platelets than recipients of non-leukocyte-depleted products. Leukocyte depletion can be performed immediately after collection of a unit of whole blood before its storage (prestorage leukodepletion) or just before the transfusion of the blood product to a recipient, after its storage (poststorage leukodepletion). However, the most appropriate time for the leukodepletion of blood products has not been established. The present study was undertaken to establish an animal model of allogeneic platelet refractoriness, and to compare the effect of prestorage and poststorage leukodepletion on the frequency of refractoriness to allogeneic donor platelets. In this model, two strains of rabbits were used: California Black rabbits were used as blood donors, while New Zealand White rabbits were used as recipients. Eight weekly infusions of nonleukodepleted allogeneic fresh blood resulted in an allogeneic platelet refractoriness rate of 91.2% (31/34). The prestorage leukodepletion of the donor blood was associated with a significantly higher allogeneic platelet survival and lower refractoriness rate (33.3%) to allogeneic platelets than poststorage leukodepletion (66.7%). Furthermore, the data suggest that cell-free plasma products are capable of inducing refractoriness to allogeneic donor platelets; the stored plasma having a greater likelihood of inducing such refractoriness than fresh plasma. Thus, these data provide evidence that the prestorage leukodepletion of allogeneic donor blood is associated with a lower frequency of refractoriness and better allogeneic platelet survival than poststorage leukodepletion.

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PLATELET TRANSFUSIONS are a well-established efficacious treatment for patients with thrombocytopenic bleeding due to bone marrow failure. However, the frequent occurrence of alloimmunization and refractoriness to random donor platelets often limit the effectiveness of platelet transfusion support, particularly in multi-transfused recipients. Published data suggest that 30% to 70% of multi-transfused individuals become refractory to random donor platelets. The exact incidence of platelet alloimmunization resulting in refractoriness is difficult to ascertain, because different criteria have been used to define platelet refractoriness. Some investigators have used posttransfusion platelet increments, some laboratory evidence of alloimmunization, while others have used both criteria. Also, there are considerable differences of opinion about which laboratory tests of alloimmunization are optimal. Clinical studies are also difficult to evaluate due to the presence of confounding factors that may contribute to inadequate platelet recovery after transfusion. These factors include bleeding, fever, sepsis, disseminated intravascular coagulation, and splenomegaly; these are factors that are often present in patients who need platelet support. The establishment of an animal model that might parallel the human situation would be an extremely useful model that could be used objectively to quantitate allogeneic platelet survival. Furthermore, such a model could be used to distinguish between refractoriness due to alloimmunization from that due to other mechanisms. Such an animal model would also be extremely useful to evaluate new approaches to reduce or eliminate factors causing refractoriness to random allogeneic donor platelets.

Recent data suggest that the leukocyte depletion of blood products may be effective in preventing or delaying the development of refractoriness to random donor platelets. These studies have suggested that patients receiving leukocyte-depleted blood products are less likely to become refractory to random donor platelets than recipients receiving non-leukocyte-depleted products. Effective methods for the removal of leukocytes from blood products have recently become available. Moreover, some of these methods appear capable of reducing leukocyte contamination by several orders of magnitude; however, their cost effectiveness has yet to be established. Leukocyte depletion can be performed immediately after the collection of whole blood, before its storage (prestorage leukodepletion), or just before the transfusion of a blood product (poststorage leukodepletion). This study was undertaken to establish an animal model of allogeneic donor platelet refractoriness, and to ascertain the efficacy of prestorage versus poststorage leukodepletion of blood products, in reducing the frequency of refractoriness to allogeneic donor platelets.

MATERIALS AND METHODS

Animal model to study refractoriness to allogeneic donor platelets. Two different strains of outbred rabbits were used for these studies. The blood donors were California Black (CB) rabbits weighing 2.5 to 3.5 kg; New Zealand White (NZW) rabbits, also weighing 2.5 to 3.5 kg, were the recipients. The rabbits were purchased by the Animal Care Facility at McMaster University Medical Centre. Only male animals were used in all experiments to avoid the potential confounding problem of alloimmunization due to pregnancy. Blood was collected from at least 10 CB rabbits using...
the main auricular artery, 15 mL from each rabbit into CPD (7 vol whole blood to 1 vol CPD). Blood from each animal was pooled, mixed, and then 15-mL aliquots were transfused via a marginal ear vein to recipient NZW animals. After either 4 or 8 weeks of allogeneic whole blood transfusions, from the same donors, a platelet survival estimate was performed on each recipient animal using pooled fresh $^{51}$Cr-labeled platelets from CB rabbits as previously described. The platelet survival time for each recipient animal was calculated using a standard-assisted computer technique that determined, by the least-squares method, the mean platelet survival as a y-function. The platelet survival time results were compared with that of a retrospective group of 26 nontransfused NZW male rabbits that had a mean survival time of fresh pooled platelets of 58.5 hours (SD 8.3 hours).

**Leukodepletion of fresh blood.** Whole blood was collected, as described above, from at least 10 CB rabbits. Fresh whole blood was pooled, mixed, and divided into two aliquots. One aliquot was leukodepleted using a second-generation leukocyte-depletion system (Leukotrap Red Cell Storage System; Cutter Biological, Berkeley, CA) providing at least 2 log (99.5%) leukodepletion. The other aliquot was not leukodepleted. Groups of 10 recipient NZW animals were transfused weekly, for 8 weeks with either 15 mL of the leukodepleted or 15 mL of nonleukodepleted fresh whole blood. After 8 weeks, platelet survival times were determined for both groups of recipient NZW rabbits using fresh CB rabbit $^{51}$Cr-labeled platelets from the same pool of donor animals. The platelet survival times and the refractory rates in NZW rabbits that received four weekly transfusions of whole blood had a refractory rate of 50% with a mean platelet survival time of 58.5 hours. The normal range (mean ± 2 SD) was 41.9 to 75.1 hours. Allogeneic (CB) platelet survival times of less than 41.9 hours, in the NZW recipient rabbits that had received allogeneic blood transfusions, were therefore considered to represent decreased platelet survival associated with the refractory state. The group of 10 recipient NZW rabbits that received four weekly transfusions of whole blood had a refractory rate of 50% with a mean platelet survival time of 30.4 hours, whereas the 34 NZW recipient rabbits that had been transfused weekly for 8 weeks had a refractory rate of 10.1%, with a mean platelet survival time of 22.3 hours (SD 16.1 hours). The refractory rate in the rabbits transfused for 8 weeks is statistically greater than for the rabbits that had been transfused for only 4 weeks ($P = .0001$).

**Effect of leukodepletion of allogeneic blood on the platelet survival time and refractory rate of allogeneic donor platelets.** The platelet survival times and the refractory rates in NZW recipient rabbits that were transfused with either leukodepleted or nonleukodepleted allogeneic fresh whole blood weekly for 8 weeks are shown in Table 1. Rabbis that had received leukodepleted allogeneic blood had a mean platelet survival time of 52.8 hours, whereas rabbits that had received nonleukodepleted allogeneic whole blood had a mean platelet survival time of only 22.3 hours ($P = .0001$). The refractory rate is also statistically different ($P = .0003$)

**RESULTS**

**Allogeneic platelet survival time and refractory rate in recipient NZW rabbits receiving nonleukodepleted allogeneic whole blood.** The mean platelet survival ($y$-function) time in the retrospective group of 26 normal NZW animals was 58.5 hours. The normal range (mean ± 2 SD) was 41.9 to 75.1 hours. Allogeneic (CB) platelet survival times of less than 41.9 hours, in the NZW recipient rabbits that had received allogeneic blood transfusions, were therefore considered to represent decreased platelet survival associated with the refractory state. The group of 10 recipient NZW rabbits that received four weekly transfusions of whole blood had a refractory rate of 50% with a mean platelet survival time of 30.4 hours, whereas the 34 NZW recipient rabbits that had been transfused weekly for 8 weeks had a refractory rate of 10.1%, with a mean platelet survival time of 22.3 hours (SD 16.1 hours). The refractory rate in the rabbits transfused for 8 weeks is statistically greater than for the rabbits that had been transfused for only 4 weeks ($P = .0001$).

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**Table 1. Platelet Survival and Refractory Rate of Allogeneic Rabbit (CB) Platelets in Recipient NZW Rabbits Receiving Either Leukodepleted or Nonleukodepleted Fresh Blood Weekly for Eight Weeks**

<table>
<thead>
<tr>
<th>Donor Blood Received</th>
<th>n</th>
<th>Mean Platelet Survival (95% CI)</th>
<th>Refractory Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukodepleted</td>
<td>10</td>
<td>52.8 h (SD 18.2)‡</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(39.8-65.8 h)</td>
<td></td>
</tr>
<tr>
<td>Nonleukodepleted</td>
<td>34</td>
<td>22.3 h (SD 16.1)§</td>
<td>91.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(16.7-27.9 h)</td>
<td></td>
</tr>
</tbody>
</table>

*CI, confidence intervals. ‡SD, standard deviation.
in these two groups of recipient animals, with a refractory rate of 30% in NZW recipient rabbits that had received leukodepleted blood and 91.2% in NZW animals that had received nonleukodepleted blood.

Effect of prestorage versus poststorage leukodepletion on refractoriness to allogeneic donor platelets. The leukocyte depletion of the transfused blood in these two groups of rabbits was similar; prestorage leukodepletion produced 99.5% leukodepletion (mean leukocyte count reduction: 6.738 to 0.034 \times 10^6) and poststorage leukodepletion was 99.6% effective (mean leukocyte count reduction: 5.328 to 0.022 \times 10^6/L). The numbers given are the means of 15 determinations in each category. Therefore, the two groups of animals received blood that was equivalent both in terms of leukodepletion and in terms of storage period. Allogeneic donor platelet mean survival time and refractory rates of leukodepletion and in terms of storage period. Allogeneic donor platelet mean survival time and refractory rates of leukodepletion and in terms of storage period. Allogeneic donor platelet mean survival time and refractory rates of leukodepletion and in terms of storage period. Allogeneic donor platelet mean survival time and refractory rates.

Table 2. Platelet Survival and Refractory Rate of Allogeneic (CB) Platelets in Recipient NZB Rabbits That Had Received Eight Weekly Transfusions of Prestorage or Poststorage Leukodepleted Donor Blood

<table>
<thead>
<tr>
<th>Leukodepletion Process</th>
<th>Donor Blood</th>
<th>Mean Platelet Survival (95% CI)</th>
<th>Refractory Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prestorage</td>
<td>n=15</td>
<td>54.7 h (SD 20.6) (43.3-66.1 h)</td>
<td>33.3</td>
</tr>
<tr>
<td>Poststorage</td>
<td>n=15</td>
<td>31.0 h (SD 14.4) (23.1-39.0 h)</td>
<td>66.7</td>
</tr>
</tbody>
</table>

Detection of lymphocytotoxic and platelet-related alloantibodies. Lymphocytotoxic antibodies to CB rabbit lymphocytes could be demonstrated in the serum of only 1 of 31 refractory NZW rabbits tested. None of 16 nonrefractory animals tested provided any evidence of lymphocytotoxic antibodies to CB rabbit lymphocytes. Using the platelet radioimmunoprecipitation technique, the sera of 12 of 31 refractory NZW animals were shown to contain antibodies to CB rabbit platelet antigens. None of the sera of the 16 nonrefractory animals tested positive for the presence of antibodies to CB rabbit platelet antigens. This difference in platelet antibody incidence between refractory and nonrefractory animals is statistically significant (P = .004).

DISCUSSION

The available clinical data in humans suggests that the leukodepletion of blood products is effective in reducing the rate of refractoriness to random donor platelets. However, many questions regarding leukodepletion remain unanswered. One unanswered question is whether the leukodepletion should be performed before storage (prestorage leukodepletion) or just before transfusion after storage (poststorage leukodepletion). The results from these animal studies clearly demonstrate that most recipient animals become refractory to allogeneic platelets after eight weekly transfusions of allogeneic whole blood. The refractory rate in recipient NZW rabbits was somewhat dependent on the amount of blood transfused. Animals transfused with allogeneic blood for 8 weeks had a refractory rate of 91.2%, whereas those transfused for 4 weeks had a refractory rate of 50%. The latter refractory rate is similar to that seen in humans; however, for the purposes of examining the effect of the various parameters of leukodepletion, we used recipient animals that were transfused for 8 weeks. This was done to maximize the frequency of the refractory rate, and thus maximize the effect of the leukodepletion. Thus, we were able to provide data that clearly demonstrate that the leukodepletion of allogeneic fresh whole blood significantly reduces the frequency of allogeneic donor platelet refractoriness and increases their in vivo survival. Furthermore, the

Table 3. Changes in Leukocyte Counts During the Storage of CB Rabbit Whole Blood (n = 15) for One Week

<table>
<thead>
<tr>
<th>Blood Storage Time (d)</th>
<th>Mean (SD) Leukocyte Count \times 10^6/mL (95% CI)</th>
<th>% Reduction in Leukocyte Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.74 (SD 0.80) (6.3-7.2)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6.24 (SD 0.93) (5.7-6.8)</td>
<td>7.4</td>
</tr>
<tr>
<td>7</td>
<td>5.33 (SD 0.94) (4.8-5.9)</td>
<td>20.9</td>
</tr>
</tbody>
</table>

Table 4. Platelet Survival and Refractory Rate of Allogeneic Platelets in Recipient NZB Rabbits That Had Received Either Fresh Plasma or Stored Plasma Weekly for Eight Weeks

<table>
<thead>
<tr>
<th>Product Transferred</th>
<th>n</th>
<th>Mean Platelet Survival (95% CI)</th>
<th>Refractory Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh plasma</td>
<td>12</td>
<td>56.1 h (SD 13.8) (47.4-64.9 h)</td>
<td>16.7</td>
</tr>
<tr>
<td>Stored plasma</td>
<td>18</td>
<td>40.1 h (SD 13.4) (33.4-46.7 h)</td>
<td>61.1</td>
</tr>
</tbody>
</table>
present results show clearly that the prestorage leukodepletion of allogeneic donor blood is associated with a reduced frequency of allogeneic platelet refractoriness and a higher in vivo survival, compared with poststorage leukodepletion.

Little is known about the major histocompatibility complex (MHC) in rabbits. Furthermore, both strains of rabbits used in the present study are outbred. For this reason we transfused pooled allogeneic blood to recipient animals. The amount of blood infused weekly (15 mL) represents the equivalent of approximately 1 U of whole blood infused into an adult human. To partially validate our animal model, we were able to demonstrate that changes in allogeneic platelet survival correlated somewhat with the development of specific platelet or lymphocytotoxic antibodies in recipient animals who became refractory to allogeneic donor platelets. We used two different approaches to ascertain the presence of antibodies. Using the complement-mediated lymphocytotoxicity test, antibodies to CB rabbit lymphocytes could be demonstrated in only 1 of 31 refractory animals tested. Using the platelet radioimmunoprecipitation technique, the sera of 12 of 31 recipient animals that were refractory to allogeneic donor platelets were shown to contain antibodies to allogeneic platelet antigens. In none of the sera of 16 nonrefractory animals tested could we demonstrate the presence of antibody using either technique. Thus, the incidence of antibodies in refractory rabbits was significantly higher \( P = .004 \) than in nonrefractory animals. Further characterization of these antibodies is in progress. Nonetheless, these preliminary antibody data suggest that the incidence of antibody positivity reflects the technique used, similar to that seen in humans, and also that the refractory state in recipient animals is immune mediated.

The present data indicate that during the storage of rabbit whole blood for 1 week the leukocyte count decreases by over 20%. These results are essentially similar to those observed during the storage of human blood.\(^24\)\(^25\) Thus, these data suggest that leukocytes can release soluble antigens, or microparticles containing antigens, into the plasma during storage and that these antigens escape leukodepletion. Similarly, HLA antigens have been demonstrated in stored human plasma.\(^26\) The data obtained in this study, with the transfusion of poststorage leukodepleted blood, are consistent with this hypothesis. Furthermore, the results obtained from the groups of rabbits that received allogeneic plasma infusions suggest that refractoriness to allogeneic platelets may be induced by non-intact leukocytes.

To our knowledge this is the first demonstration of the advantages of the prestorage leukodepletion of blood products over poststorage leukodepletion. However, data from animal experiments cannot necessarily be extrapolated to the human situation. Therefore, we recommend that properly designed prospective, randomized clinical studies be conducted to ascertain whether the superiority of prestorage leukodepletion over poststorage leukodepletion is also true for humans.

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