A randomized study was performed in 54 thrombocytopenic patients with acute leukemia. Alloimmunization of recipients of random multiple-donor platelet concentrates (MD group) was compared to that of patients receiving random single-donor platelets (SD group). In the SD patients, formation of alloantibodies (mostly anti-HLA) occurred less frequently (<0.002), after a longer time period (<0.002), and after a higher number of transfusions (<0.005) as compared to MD patients. SD patients also became refractory to random platelets less frequently (<0.005), after a longer time period, and after a higher number of transfusions (<0.02). In SD patients, the increments after the first and the last transfusion were in the same range, whereas in MD patients, the 1-hr (<0.001) and the 24-hr (<0.025) increments decreased from the first to the last transfusion. Thus, the use of random SD platelet transfusions postponed alloimmunization.

The same clinical and laboratory criteria for platelet support were applied to all patients, i.e., platelet transfusion within 12 hr if (1) morning counts were below 5,000/cu mm, or (2) morning counts of 5,000-10,000/cu mm together with at least minimal new signs of hemorrhage and/or fever >38°C, or (3) morning counts above 10,000/cu mm with major bleeding. Leukocyte-poor packed red cells were transfused to maintain the hemoglobin in the range of 6.0-9.0 g/dl. In 92% of the patients of both groups, the same two therapy protocols were applied for remission induction (protocol SAKK 30/78 for AML). 23 MD and 21 SD patients; a pilot study protocol of induction treatment consisting of anthracyclines, cyclophosphamide, bleomycin, vincristine, and prednisone for adult ALL: 2 MD and 2 SD patients).

The study was terminated for an individual patient if the following events occurred: (1) induction chemotherapy completed (MD group: 8 patients, SD group: 16 patients); (2) death during induction treatment (MD group: 4 patients, SD group: 2 patients); (3) application of granulocyte transfusions (MD group: 1 patient, SD group: 4 patients); (4) technical breakdown of cell separator (SD group: 1 patient); (5) clinical refractoriness to random donor platelet transfusions necessitating subsequent HLA-matched single-donor platelet transfusions (MD group: 14 patients, SD group: 4 patients).

The study period is defined as the number of days from the first blood component transfusion to the last platelet transfusion.

Blood Component Preparation
Platelet concentrates for patients of the MD group were prepared according to the standard acid citric dextrose (ACD) split method at 20°C, starting from 500 ml freshly collected whole blood in plastic bags (Double Platelet Pack, Fenwal, FPR 1212). The number of...
platelets given to MD patients was determined in 60 of the 148 transfusions. For interpretation of the increments of the remaining 88 MD transfusions, a representative mean platelet count per concentrate was established. Therefore, platelet counts were determined in an additional 105 pooled platelet preparations, each of them consisting of 5-8 (mean 7) platelet concentrates. The mean amount of platelets per concentrate obtained from these determinations was 0.85 x 10^11. Given the relatively small variability in actual platelet counts of pooled preparations, 0.86 x 10^11 platelets were designated as one platelet unit.

Platelet collection from single donors was performed by use of the Haemonetics’ 30 blood cell processor with ACD formula A (USP) as anticoagulant. The number of platelets collected was determined in all single-donor preparations and revealed 5.9 ± 1.2 x 10^11 platelets per preparation. Mean leukocyte content per 10^11 platelets was higher in single-donor preparations as compared to platelet concentrates (8.9 x 10^6 versus 2.0 x 10^7). Platelets collected by both methods showed similar posttransfusion recoveries and survival times as analyzed previously by chromium-51-labeled autologous retransfusion experiments. All platelet preparations were kept at room temperature and administered within 6 hr of collection.

For preparation of leukocyte-poor red cells, standard methods were used, allowing an elimination of over 95% of the leukocytes in the red cells.

Platelet Counts
Platelet counts were done prior to as well as 1 and 24 hr after transfusion. Routinely, a Coulter Counter S Plus was used. Counts below 50,000/cu mm were determined by manual phase-contrast microscopy counting. The high platelet counts of platelet concentrates or single-donor preparation (usually > 1 x 10^11/cu mm) were analyzed after dilution of 1.0 ml of the platelet suspension by 4.0 ml of 0.9% NaCl.

Collection of Recipient Sera
Sera from all patients were collected at study entry and at intervals of 2–3 wk throughout the study period. In all but 3 patients surviving the study period, an additional final serum was obtained 6–30 (mean 14.1) days after the last platelet transfusion. All sera were kept at –80°C until testing.

Histocompatibility Testing and Antibody Screening
Histocompatibility testing was performed by the standard microlymphocytotoxicity assay. Lymphocytotoxicity tests between the patient sera and the lymphocytes of a test panel were performed by standard methods at room temperature and 60 min incubation time following addition of complement. An individual test was designated positive if at least 50% of the cells in the well were killed. Appropriate negative and positive controls were always included. The lymphocyte test panel consisted of 44 “fullhouse-type” freshly prepared lymphocyte suspensions. Sixteen specificities and splits of HLA locus A and 29 of locus B, covering over 95% of the presently known specificities, were represented in the test panel.

Serum antibodies of the IgG and IgM class binding to platelets were determined in a modified radioimmunoassay in which protein A was replaced by 125I-labeled rabbit anti-human IgG and IgM antibodies purified by affinity chromatography. Two platelet suspensions, each of them containing equal amounts of platelets from 20 donors of blood group 0, were used as antigens. A test was considered positive if the counts per minute (cpm) exceeded the mean ± 3 SD (i.e., mean ± 30%) of 30 normal sera tested.

Evaluation of Transfusion Responses
Transfusion responses were expressed as “corrected platelet increments” in order to evaluate the posttransfusion increment independently of size of the recipient and various doses of transfused platelets. Platelet increments 1 hr, as well as 24 hr, after transfusion were therefore multiplied by body surface area (sq m) and divided by the number of “platelet units” transfused. The number of “units” in single-donor preparations was calculated by dividing the total amount of platelets collected by 0.86 x 10^11, i.e., the mean value of our platelet concentrates (see above).

A patient was designated refractory to random donor platelets subsequent to the third incompatible platelet transfusion response. A platelet transfusion was determined to be incompatible: (1) if the corrected 24-hr increment was less than 2,500 sq m/cu mm/unit in patients without a nonspecific mechanism of accelerated platelet consumption; or (2) in the presence of a nonspecific consumption mechanism, if the corrected 1-hr increment was less than 4,000 sq m/cu mm/unit in addition to a corrected 24-hr increment of less than 2,500 sq m/cu mm/unit. A nonspecific platelet consumption mechanism was assumed to be present if: (1) coagulation tests demonstrated a disseminated intravascular coagulation, or (2) clinical signs of septicemia with positive blood cultures were present, or (3) the spleen was enlarged to at least 5 cm below the left costal margin.

Statistical Analysis
For comparison of the two groups, Student’s t test was used when no considerable deviation from symmetric distribution was observed. When data fell in different classes, the chi-square test for contingency tables was applied. To compare the time (or the number of transfusions) needed to become refractory (or produce antibody), a life table analysis according to Kaplan and Meier was performed. These analyses were compared by the chi-square test according to Peto and Pike.

RESULTS
Comparison of Experimental Groups (Table 1)
Fifty-four patients with acute leukemia were randomized, 27 to receive multiple donor platelet concentrates (MD group: 24 ANLL, 3 ALL) and 27 to be substituted by single-donor platelet transfusions (SD group: 23 ANLL, 4 ALL). The two groups were comparable with respect to age, sex, diagnosis, and intensity of chemotherapy. In addition, previous pregnancies were equally distributed among both groups: 3 MD and 4 SD patients had one pregnancy, 5 MD and 6 SD patients had 2 pregnancies, and 4 MD and 3 SD patients had 3 or 4 pregnancies. The mean number of platelet transfusions, the total amount of platelet units and of red cell units transfused was similar in both groups. Due to the lower number of SD patients alloimmunized during study, the mean study period was somewhat longer in the SD group, but the difference was not significant. Nonimmunologic factors known to decrease posttransfusion platelet recovery were regularly recorded in all patients. They were equally distributed among the two groups.
Table 1. Comparison of the Two Study Groups

<table>
<thead>
<tr>
<th>Feature</th>
<th>MD Group</th>
<th>SD Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>49.8</td>
<td>40.9</td>
</tr>
<tr>
<td>Range</td>
<td>24-68</td>
<td>17-71</td>
</tr>
<tr>
<td>No. of female patients</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Mean number of platelet transfusions per patient (± 1 SD)</td>
<td>5.5 (± 3.0)</td>
<td>6.0 (± 3.0)</td>
</tr>
<tr>
<td>Mean number of platelet units per patient (± 1 SD)</td>
<td>36.6 (± 20)</td>
<td>34.3 (± 18)</td>
</tr>
<tr>
<td>Mean number of red cell units per patient (± 1 SD)</td>
<td>10.7 (± 5.5)</td>
<td>12.8 (± 6.0)</td>
</tr>
</tbody>
</table>

Study period (days)

<table>
<thead>
<tr>
<th>Feature</th>
<th>MD Group</th>
<th>SD Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean and range</td>
<td>32 (3-136)</td>
<td>9 (4-159)</td>
</tr>
<tr>
<td>No. of patients</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>20 days</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>21-40 days</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>40 days</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>No. of episodes of DIC*</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Septicemia†</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Splenomegaly‡</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

*Disseminated intravascular coagulation (defined as a drop of fibrinogen below 100 mg/dl and the presence of fibrinogen degradation products > 4 mg/dl).
†Clinical signs of septicemia with positive blood cultures.
‡Spleen enlarged to at least 5 cm below left costal margin.

Antibody Screening

The frequency and rate of alloimmunization in terms of the development of detectable lymphocytotoxic antibodies was investigated in both groups by use of a large panel of lymphocytes. Sera obtained prior to the first transfusion were consistently negative with all panel lymphocytes. While receiving prophylactic platelet support, 14 of 27 patients of the MD group, as compared to 3 of 27 of the SD group, developed lymphocytotoxic antibodies detectable in at least one serum (p < 0.001). In 15 of the 17 cases with lymphocytotoxic antibodies, the strength of the individual cytotoxic reaction was high (more than 75% of cells in the well were killed in the majority of the panel lymphocytes). In addition, the sera showed positive reactions with a remarkably high percentage of the panel lymphocytes in most cases (Fig. 1, upper graph).

Sera of all patients with negative lymphocyte cytotoxicity tests (MD group: 13; SD group: 24) were further investigated for the presence of platelet-binding IgM and IgG antibodies. IgM antibodies were not detected in any of these 37 patients, but one patient of each group demonstrated platelet-binding IgG.

Thus, 15 patients of the MD group developed detectable alloantibodies while receiving multiple-donor platelet transfusions. In the SD group, alloantibodies could be demonstrated in only 4 patients (p < 0.002; Table 2). In the MD group, 10 of 12 women with previous pregnancies formed antibodies, as compared to 2 of 13 in the SD group (p < 0.001). In patients without previous pregnancies, 5 of 15 in the MD group and 2 of 14 in the SD group developed antibodies (p < 0.2).

In order to evaluate the percentage of patients developing antibodies as a function of time or as a function of the number of platelet transfusions, a Kaplan-Meier life-table analysis was done. The median number of days from the initial transfusion to the first serum demonstrating antibodies was significantly shorter in the MD group as compared to the SD group (p < 0.005; Table 2, Fig. 2). Likewise, the MD group received a significantly smaller median number of platelet transfusions (p = 0.001) until antibodies could be detected.

Specificity of Lymphocytotoxic Antibodies

In 10 of the 17 patients with lymphocytotoxic antibodies, the specificity of these antibodies could be
Table 2. Overall Incidence, Frequency, and Rate of Antibody Formation and of Refractoriness to Random Donor Platelet Transfusions

<table>
<thead>
<tr>
<th></th>
<th>MD Group</th>
<th>SD Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients with detectable antibodies*</td>
<td>15</td>
<td>4</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Number of days from first transfusion to antibody detection†</td>
<td>23</td>
<td>NR†</td>
<td>&lt;0.006</td>
</tr>
<tr>
<td>95% Confidence limit</td>
<td>18-78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of platelet transfusions until antibody detection†</td>
<td>6</td>
<td>NR</td>
<td>0.001</td>
</tr>
<tr>
<td>95% Confidence limit</td>
<td>4.2-8.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients refractory*</td>
<td>14</td>
<td>4</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Number of days from first transfusion to refractoriness†</td>
<td>21</td>
<td>NR</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>95% Confidence limit</td>
<td>15-90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of platelet transfusions until refractoriness‡</td>
<td>6.3</td>
<td>NR</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>95% Confidence limit</td>
<td>4.9-7.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Student's t test.
†Kaplan-Meier life table analysis.
‡NR, median value not yet reached.

Further investigated by additional selected lymphocytotoxicity tests. Therefore, the strongest positive serum of each of these patients was tested against several ‘full-house-typed’ lymphocytes identical to the HLA-A, B type of the respective patient. Despite the high percentage of positive tests with the (HLA-nonidentical) lymphocytes of the test panel (Fig. 1, upper graph), these tests with HLA-A, B identical lymphocytes were consistently negative (Fig. 1, bottom).

Platelet Transfusion Responses

Trends in the 1-hr, as well as the 24-hr, increments for serial MD and SD transfusions were analyzed by comparing the posttransfusion increments after the first and the last transfusion of each patient (Table 3). Patients who received only one platelet transfusion (MD group: 2; SD group: 3) and transfusions given in the presence of overt septicemia or disseminated intravascular coagulation (DIC) (MD group: 5; SD group: 6) were not included in this analysis. The mean 1-hr, as well as 24-hr, increments observed after the first transfusion were similar in both groups. By contrast, mean 1-hr and 24-hr increments observed after the last transfusion were significantly lower in the MD group as compared to the SD group (p < 0.01). In addition, patients of the MD group demonstrated a significant decrease in the 1-hr and 24-hr increment

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Fig. 2. Kaplan-Meier life table plot of frequency and rate of (1) antibody formation as a function of time (A) or number of platelet transfusions (B), and (2) of refractoriness as a function of time (C) or number of platelet transfusions (D). Ordinate: Probability (%) of not forming antibodies (A, B), respectively of not becoming refractory (C, D). MD group (—); SD group (—).
In all cases, posttransfusion increments were compatible according to our study protocol and analyzing serial serum samples obtained throughout the study period, a significant correlation of in vivo and in vitro parameters of alloimmunization was observed (Table 4). Thirty patients demonstrated no refractoriness to random donor platelets and no detectable antibodies (MD group: 9 patients, SD group: 21 patients). On the other hand, 13 patients were characterized by refractoriness together with the development of antibodies (MD group: 11 patients, SD group: 2 patients).

In 6 patients, diverging in vivo and in vitro results were found. Five of them (MD group: 3, SD group: 2) were refractory to random donor platelets, but lymphocytotoxicity tests as well as platelet-binding antibody tests were negative. In 4 of them, one or several nonimmunologic platelet consumption mechanisms could be responsible for the unsatisfactory random platelet responses, whereas in 1 MD patient, the transiently poor platelet increments are unexplained. The remaining patient demonstrated positive lymphocytotoxicity tests following six red cell transfusions and prior to the first (and incompatible) MD platelet transfusion. Subsequently, no lymphocytotoxic antibodies were detected in several serum samples and all additional MD platelet transfusions were compatible.

In five patients not included in Table 4 (MD group: 3; SD group: 2), antibody formation occurred toward the end of the study period of the individual patient, when platelet support was not needed anymore and therefore refractoriness could not be properly evaluated. In one patient from each group, antibodies were detectable for the first time on the day of their last transfusion. Subsequently, no lymphocytotoxic antibodies were detected in several serum samples and all additional MD platelet transfusions were compatible.

Correlation of In Vivo Refractoriness and In Vitro Antibody Detection

Applying the strict criteria for refractoriness outlined in our study protocol and analyzing serial serum samples obtained throughout the study period, a significant correlation of in vivo and in vitro parameters of alloimmunization was observed (Table 4). Thirty patients demonstrated no refractoriness to random donor platelets and no detectable antibodies (MD group: 9 patients, SD group: 21 patients). On the other hand, 13 patients were characterized by refractoriness together with the development of antibodies (MD group: 11 patients, SD group: 2 patients).

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In five patients not included in Table 4 (MD group: 3; SD group: 2), antibody formation occurred toward the end of the study period of the individual patient, when platelet support was not needed anymore and therefore refractoriness could not be properly evaluated. In one patient from each group, antibodies were detectable for the first time on the day of their last (and incompatible) platelet transfusion. In the remaining 3 patients, antibodies were observed for the first time in the final serum obtained 10–14 days after the last transfusion.

Correlation of in vivo and in vitro parameters of alloimmunization can also be demonstrated by the comparison of platelet increments observed after the last transfusion in patients with and without detectable antibodies. This analysis was performed in all 45 patients who received at least two transfusions and in whom nonimmunologic consumption mechanisms were absent at the time of the last platelet transfusion. The mean value of the corrected 24-hr increment of patients with antibodies was lower as compared to the mean value of patients without detectable antibodies (p < 0.001, Fig. 3).
DISCUSSION

Repeated transfusions of platelet concentrates obtained from randomly selected donors often result in alloimmunization, as can be demonstrated by the development of lymphocytotoxic antibodies in 35%–70% of these patients.1–3,13–17 In the MD group in our study, a comparable proportion of recipients of random donor concentrates formed such antibodies (55%). Women with previous pregnancies showed a particularly high percentage of antibody formation after MD platelet transfusions. This high rate of alloimmunization in previously untransfused leukemic patients occurred despite the concomitant application of aggressive remission induction therapy. In accordance with others,3,16 antibodies were detectable after a small number of platelet transfusions and within a few weeks in the majority of patients becoming alloimmunized. In contrast to the MD group, only 11% of the SD group developed lymphocytotoxic antibodies.

Using a large panel of HLA-A, B-typed lymphocytes, we could show that lymphocytotoxic sera of alloimmunized patients were usually reactive with a high proportion of HLA-A, B-nonidentical lymphocytes. Monospecific anti-HLA-A, B antibodies were not observed in either recipients of MD or SD platelets. The polyspecificity of the lymphocytotoxic antibodies was directed toward the HLA-A, B antigens: The strongest positive sera—broadly reactive with HLA-A, B-nonidentical lymphocytes—were consistently negative with “full-house-typed” lymphocytes identical to the HLA loci A and B of the respective patient. Moreover, good posttransfusion platelet increments were obtained with HLA-A, B-identical platelet transfusions in patients highly refractory to random platelets. Our results confirm the outstanding role of HLA antigens of loci A and B with respect to long-term platelet support1,4,14,17 and indicate that clinically relevant platelet-specific alloantibodies are infrequent in recipients both of MD and SD random donor platelet transfusions.

In accordance with most authors,1–3,14,16–18 a good correlation of the presence of lymphocytotoxic antibodies and low posttransfusion platelet increments was observed in the majority of our patients. Patients with detectable lymphocytotoxic antibodies consistently showed incompatible posttransfusion increments to random platelets. On the other hand, the relationship of negative lymphocytotoxicity tests and compatible transfusion responses throughout the entire study period was less consistent. In 14 patients, at least 1 incompatible posttransfusion increment was observed despite negative lymphocytotoxicity as well as platelet-binding antibody tests. In all but one of these patients, nonimmunologic mechanisms known to accelerate platelet consumption12,17,19 were concurrently present as a possible explanation for the mostly transient poor platelet transfusion responses. However, the often complex situation of a severely ill leukemic patient...

Fig. 3. Corrected 24-hr posttransfusion platelet increments after the last transfusion in patients with (left) and without (right) antibodies. (3) MD patients; (A) SD patients; (—) mean values. (1) Patients developing antibodies 13 and 14 days after the last platelet transfusion.
needing supportive care makes it sometimes difficult to judge if a patient is (transiently) unresponsive to random donor platelets purely due to alloimmunization or rather due to nonimmunologic platelet consumption mechanisms, or both.

Our results indicate that in untransfused leukemic patients, both antibody formation and refractoriness can at least be postponed by the use of random single donors instead of random multiple donors, thus allowing more aggressive chemotherapy. Similar results have recently been published, although these authors observed no difference in the frequency of antibody formation in patients receiving single-donor or multiple-donor platelet transfusions. This may be due to the small number of patients and platelet transfusions.

So far, the superiority of our single-donor transfusion approach has only been demonstrated in nontransfused patients and for a limited period of time. Female patients with previous pregnancies benefited particularly from this transfusion policy. It remains to be determined whether comparable results can be obtained in patients preimmunized by previous transfusions, and in nonleukemic patients needing platelet support for a much longer period of time.

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Delayed alloimmunization using random single donor platelet transfusions: a prospective study in thrombocytopenic patients with acute leukemia

J Gmur, A von Felten, B Osterwalder, H Honegger, A Hormann, C Sauter, K Deubelbeiss, W Berchtold, M Metaxas, G Scali and PG Frick