Hematopoietic Graft Detected by a Change in ABO Group

By William R. Bronson, Mary H. McGinniss and Edward E. Morse

For the past two years the Medicine Branch of the National Cancer Institute has employed granulocyte transfusions in an attempt to provide phagocytic cells to combat septicemia in the leukopenic phase of acute leukemia. For this purpose, donors with chronic myelogenous leukemia were used to obtain adequate numbers of polymorphonuclear cells. A group O boy with acute lymphocytic leukemia who underwent such treatment was found to be transiently of group AB after receiving white cell transfusions from an AB donor. The number of AB cells in the recipient could not be accounted for by transfused cells. Evidence will be presented to indicate that hematopoietic cells from the peripheral blood of the AB donor were grafted to the O recipient.

Methods

White blood cell and red blood cell counts were determined with an electronic Coulter Counter, platelets were counted by the method of Brecher and Cronkite, and reticulocytes with the new methylene blue technic. Hemoglobin was measured by the cyanmethemoglobin method and serum bilirubin was determined with an ultramicro modification of the technic of Malloy and Evelyn. White cell-rich and platelet-rich plasmas were prepared by plasmapheresis. Red cell counts were not done on the material transfused; however, the average red cell count in a subsequent series of 10 consecutive determinations was 375,000 per mm.\(^3\) (210,000-450,000) for white cell-rich plasma, and in another series, 20,000 per mm.\(^3\) (13,000-22,000) for platelet-rich plasma. The maximum values were used to calculate the volume of red cells in the out-of-group transfusions received by the patient.

The percentages of AB and O cells in the patient’s red blood cell population were determined by indirect differential agglutination. His red blood cells were washed 3 times with sterile 0.85 per cent saline, and a 2.0 per cent cell suspension was approximated. Exactly 0.05 ml. of this suspension was transferred to each of three tubes. Five-hundredths of a ml. of a commercial, high titered anti-A was added to the first, 0.05 ml. of anti-B to the second, and 0.05 ml. of saline to the third tube which served as a blank. Each tube was incubated at 22 C. for 10 minutes with occasional shaking and then centrifuged for 15 seconds in a Clay-Adams Serofuge. The supernate was then carefully removed without disturbing the agglutinates, diluted 1:20 in a white cell pipette, and counted in a Max Levy chamber. Two counts were made on each sample and the results averaged. The blank count minus the count of cells not agglutinated by anti-A or anti-B represents the number of AB cells in the sample.*

The cells not agglutinated by anti-A and anti-B were agglutinated with an anti-H serum diluted to the point where it reacted with group O cells alone, thus demonstrating that the anti-A or anti-B effectively removed the AB cells, leaving only O cells in the supernate. The error of the red cell counts was calculated by the Berkson formula. From the Clinical Center and National Cancer Institute, National Institutes of Health, Bethesda, Md.

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*With the same commercial antisera, less than 0.05 per cent of the donor’s A\(_1\)B cells were left unagglutinated; consequently, no correction was made for unagglutinated AB cells.
The patient's anti-A and anti-B titers were determined in parallel by serial dilutions of his serum in 0.85 per cent saline, the end point being the last dilution showing microscopic agglutination.

The direct antiglobulin tests were done using sera from five different manufacturers in parallel on each sample. The agglutination was scored by the method of Race and Sanger\(^{12}\) and the results of the five tubes averaged to give the final score.

An eluate was prepared by the rapid heat technic of Marcuse.\(^{13}\) Four drops of saline were added to 0.6 ml. of the 5-times washed packed RBC's and placed at 56°C for 5 minutes. After spinning at 3000 rpm for 2 minutes in prewarmed cups, the eluate was removed and tested with known group O, A\(_2\), and B cells.

Saliva obtained from the donor and the recipient was tested by the technic of Dunsford and Bowley to determine secretor status.\(^{14}\) Chromosome studies were done by the method of Tjio and Whang.\(^{15}\)

**Clinical History (F. J. K.)**

Acute lymphocytic leukemia was diagnosed in this 11-year-old Caucasian patient on September 30, 1960. On October 13, 1960, prior to any chemotherapy or transfusions, he was group O. All subsequent care including transfusions was received at this hospital. He died in hematologic relapse and with widespread pseudomonas infection 2 years later, on October 11, 1962. His clinical course and the antileukemic agents used are outlined in figure 1, and the period from early October to late November, 1961, encompassing the blood group change, is covered in detail in figure 2.

Admission to the hospital on October 1, 1961 was necessitated by a severe febrile illness, later diagnosed by x-ray as a bilateral bronchopneumonia, accompanied by hematologic relapse with severe leukopenia. Intravenous methotrexate was begun on October 5. Three days later a series of white cell transfusions was initiated. On October 8, just prior to these transfusions, his blood group again was found to be group O. A group AB patient with chronic myelogenous leukemia was selected as the donor for three of the four white cell preparations,* while the fourth was from a group O donor also with chronic myelogenous leukemia. The material transfused on October 11 was a white blood cell concentrate suspended in 50 ml. of saline and tagged with 0.01 per cent acridine orange, rather than white cell-rich plasma.\(^\dagger\) This was the last transfusion received from an AB donor. The volumes, white blood cell counts, platelet counts, and calculated red cell volume for the out-of-group preparations are listed in table 1.

The patient remained febrile until October 23. His white blood cell count then approached normal, his hemoglobin remained at about 8.0 Gm. per cent, and his platelet count rose above normal and reached the very high level of 2 x 10\(^6\) on October 30. No blood grouping was performed during this period. He was discharged from the hospital on October 27. Daily oral methotrexate was started on November 3, but was stopped on November 13 because of depression of white blood cell and platelet counts. During this interval he felt well and was considered to be in remission.

On November 17, he was admitted to the hospital with a febrile illness which responded to antibiotic therapy. On the day of admission his blood was group AB and further testing showed over 50 per cent of his red cell population to be AB. This was the first time since October 8 that the patient's blood group was determined. Within 24 hours his hemoglobin dropped from 8.1 Gm. per cent to 6.4 Gm. per cent and the proportion of AB cells fell from 56 to 22 per cent. Over the next 6 days these cells completely disappeared. On November 18 the patient received 170-200 ml. of group O packed red cells.

Methotrexate therapy was resumed on December 2, 1961 and on December 7 the patients' bone marrow was normal. Subsequently, he relapsed twice. He received no further

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*The AB donor was considered a universal plasma donor since he possessed neither anti-A nor anti-B. The red cell contamination was not thought to be a threat to the kidneys.

\(^\dagger\)Acridine orange was being tested to determine its usefulness as a white blood cell tag.
non-O transfusions for the remainder of his life and his RBC's remained group O. He died on October 11, 1962, and a postmortem examination confirmed the diagnosis of acute lymphocytic leukemia.

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On two occasions prior to the white cell transfusions, the recipient's red cells were group O. In addition, both parents were group O. He secreted H substance in his saliva but not A and B.

The donor with chronic myelogenous leukemia was an A1B who secreted A, B, and H substance in his saliva. An antibody directed against group O red cells was discovered in his serum in retrospect. This anti-O was not inhibited by the H substance from the saliva of the recipient, and it was active undiluted at 22 C. and 37 C. Four to five nucleated red blood cells per 100 white cells were present in his peripheral blood, and chromosome studies demonstrated the Philadelphia chromosome (Ph1) in his bone marrow cells.

The percentage of AB cells in the patient's erythrocyte population from November 17 to November 24, 1961 are listed in table 2. The AB cells fell from 56 to 0 per cent over a 7-day period. The error involved in these determinations is a function of the number of red cells counted. For the minimum count of 236 cells, the maximum error is 9 per cent. Also listed in table 2 are the titers of anti-A and anti-B in the recipient. Prior to the white cell transfusions the isoagglutinins were in the normal range. Shortly thereafter they were depressed, and during the time when AB cells were demonstrated, anti-A was absent in certain instances while anti-B was lowered. The titers rose following disappearance of the AB cells, and eventually displayed immune characteristics. The recipient's direct antiglobulin test was positive while AB cells were disappearing, and an eluate prepared from the November 20 sample possessed activity against group A and group B cells but not against group O cells. Additional red cell antigens, possessed by the donor but not by the recipient prior to transfusion, were investigated. However, the two antibodies used (anti-Kell and anti-Duffy) gave inconclusive results probably because the recipient's AB population was coated with globulin.

Anti-A and anti-B sera, neutralized with A and B substance to the point where they no longer reacted with known A1 and B cells, failed to agglutinate the November 17, 18, and 20 samples. In addition, an antiserum containing a saline-active anti-Lea that agglutinated cells known to possess the Lewis antigen did not agglutinate the November 18 specimen when tested concurrently.

To illustrate more fully the changes occurring during the period of AB cell elimination, red cell volumes were calculated using a figure of 75 ml. of whole blood per kg. of body weight, and estimated hematocrit values.* The per cent of AB and O cells had been determined by differential agglutination making it possible to calculate the contribution made by group O red cells to the total volume. These figures are listed in table 3.

*Hematocrits were estimated by multiplying hemoglobin values by a factor of 3. This implies a normal MCHC of 33, which appeared warranted since children with acute lymphocytic leukemia generally have a normochromic anemia and the same applies to the donors.
Fig. 1.—Clinical course. Complete remission indicates less than 10 per cent abnormal cells, partial remission indicates between 10 and 50 per cent abnormal cells, and relapse indicates greater than 50 per cent abnormal cells in the bone marrow.
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The patient's hemoglobin dropped from 8.1 Gm. per cent to 6.4 Gm. per cent between November 17 and 18. This was consistent with a loss primarily, if not solely, of AB cells since the percentage of AB cells in his red cell population fell from 56 to 22, a loss of approximately one-quarter of his red cell mass, or about 2 Gm. per cent of hemoglobin. Thereafter, the remaining AB cells rapidly disappeared. Chromosome studies done subsequently failed to demonstrate the Ph chromosome. Serum bilirubin did not rise during the period the AB cells were disappearing. Plasma hemoglobin levels were not measured.

DISCUSSION

Two populations of red blood cells were detected in a boy with acute lymphocytic leukemia who had been transfused with white cell-rich plasma harvested from a patient with chronic myelogenous leukemia. The boy had been of group O prior to transfusion. Six weeks after the transfusion, 56 per cent of his red cells were found to be of group AB. The AB cells were coated with anti-A and anti-B and disappeared 7 days after discovery. The titers of free isoagglutinin were depressed in the presence of the foreign cells but rose following their disappearance.

The white cell preparations transfused were from an AB donor and contained some red cells. However, the transfused cells accounted for no more than 10 per cent of the boy's calculated red cell mass. Persistence of transfused red cells, therefore, does not explain the observed change in blood group.

There are several possible explanations for this phenomenon: polyagglutinability of a portion of the patient's red cell population, modification of the patient's red cells by leukemia, adsorption of passively transfused antigens on the patient's cells, red cell mosaicism, or actual growth of donor hematopoietic tissue in the recipient. We favor the last explanation. However, the others are theoretically possible and therefore will be discussed.

Polyagglutinability of a portion of the patient's red cell population is ruled out by failure of neutralized anti-A and anti-B sera to agglutinate the November 17, 18, and 20 samples and by failure of anti Le to agglutinate the November 18 sample.

Blood group modifications in association with leukemia and other malignancies have been reported. This seems an unlikely explanation here. None of the reported changes were in group O individuals, and in none was there the acquisition of a second discrete population comparable to the A,B cells in the present case.

Passive transfer of water-soluble A and B substance in the plasma of the donor with subsequent adsorption onto the cells of the recipient is another possibility. Renton and Hancock report that group O cells transfused to group A and group B recipients become agglutinable by some group O sera and by a few group A or B sera, showing that they have taken up A or B substance. They found that the modified cells were only weakly agglutinable. Such a mechanism is unlikely here. The patient's AB population was strongly
Fig. 2.—Transfusions, anti-leukemic therapy, and hematologic data for October-November, 1961 period.
agglutinated by ordinary anti-A and anti-B sera. In addition, the only source of A and B substance was in the transfused plasma and only a limited amount was therefore available to coat the recipient's O cells. In the situation reported by Renton and Hancock, the recipient was the source of antigen which was therefore continuously available in comparatively high concentrations.

To rule out conclusively modification of blood group antigens by leukemia or passive acquisition of blood group substance, information on an additional blood group system would have been helpful. This was not possible here because the pertinent antibodies, anti-Kell and anti-Duffy, are of the incomplete type and the patient's red cells were coated with globulin.

The possibility of red cell chimerism can be eliminated since both parents were of group O.

There is much evidence to support the hypothesis that AB cells received with the white cell transfusion proliferated in the recipient, and were being rejected 6 weeks later when the blood group change was first noted. At the time of the postulated graft, the patient's immunologic mechanisms probably were impaired. The measured level of anti-A, and to a lesser extent of anti-B, was lower than normal the day following the third white cell infusion, presumably because of partial neutralization by A and B substance in the donor plasma. In addition, the patient was receiving methotrexate, which is known to increase the success of grafting bone marrow in dogs.18

The AB cells survived in this patient just over 6 weeks. Successful homologous bone marrow grafts both in dogs19 and in man20 frequently survive no longer than this, though exceptions have been reported.21,22 It may be relevant, however, that in this boy methotrexate was discontinued just 11 days before all AB cells were eliminated.

The thesis that a graft was responsible for the change in blood group in this case receives further support from observations on "takes" of chronic myelogenous leukemia cells with the Ph1 chromosome in children with acute lymphocytic leukemia. In seven of 11 children who received single or multiple white cell transfusions obtained from donors with chronic myelogenous leu-

Table 1.—Out-of-Group Transfusions

<table>
<thead>
<tr>
<th>Date Transfused</th>
<th>Donor</th>
<th>Product</th>
<th>Volume</th>
<th>WBC/mm.3</th>
<th>Platelets/mm.3</th>
<th>Calculated RBC Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct. 9, 1961</td>
<td>CML group AB</td>
<td>white cell-rich plasma</td>
<td>530 ml.</td>
<td>129,000</td>
<td>548,000</td>
<td>18 ml.</td>
</tr>
<tr>
<td>Oct. 10, 1961</td>
<td>CML group AB</td>
<td>white cell-rich plasma</td>
<td>475 ml.</td>
<td>105,000</td>
<td>445,000</td>
<td>14 ml.</td>
</tr>
<tr>
<td>Oct. 11, 1961</td>
<td>CML group AB</td>
<td>white cell concentrate</td>
<td>190 ml.</td>
<td>195,000</td>
<td>—</td>
<td>8 ml.</td>
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<tr>
<td>Oct. 17, 1961</td>
<td>normal group A</td>
<td>platelet-rich plasma</td>
<td>950 ml.</td>
<td></td>
<td></td>
<td>total A cells = 2 ml.</td>
</tr>
</tbody>
</table>

*This donor's peripheral blood contained four to five nucleated red cells per 100 white cells.
### Table 2.—Immunohematology Data

<table>
<thead>
<tr>
<th>Date</th>
<th>No. Cells in Control</th>
<th>No. Cells after Anti-A</th>
<th>% O Cells</th>
<th>No. Cells after Anti-B</th>
<th>% O Cells</th>
<th>Calc. % AB Cells</th>
<th>22 C.</th>
<th>37 C.</th>
<th>ICT*</th>
<th>Direct Coombs Score</th>
<th>Eluate</th>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>32</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td>Nov. 17, 1961</td>
<td>555</td>
<td>245</td>
<td>44</td>
<td>236</td>
<td>43</td>
<td>56, 57</td>
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<td>0</td>
<td>4</td>
<td>0</td>
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<td>18</td>
<td>476</td>
<td>337</td>
<td>71</td>
<td>409</td>
<td>86</td>
<td>29, 14</td>
<td>1</td>
<td>16</td>
<td>0</td>
<td>8</td>
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<td>20</td>
<td>464</td>
<td>417</td>
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<td>401</td>
<td>86</td>
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<td>8</td>
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<td>24</td>
<td>-</td>
<td>-</td>
<td>100†</td>
<td>-</td>
<td>100†</td>
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<td>-</td>
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<tr>
<td>Dec. 8, 1961</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>32</td>
<td>64</td>
<td>64</td>
<td>64</td>
<td>128</td>
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<tr>
<td>Apr. 4, 1962</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>32</td>
<td>128</td>
<td>128</td>
<td>128</td>
<td>0</td>
</tr>
</tbody>
</table>

*Indirect Coombs test.
†No cells agglutinated by anti-A or anti-B.
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Table 3.—Hematologic Changes during Period AB Cells Were Disappearing

<table>
<thead>
<tr>
<th>Date</th>
<th>Reticulocytes</th>
<th>Hb Gm.</th>
<th>Calc. Hct</th>
<th>Calc. Total RBC Mass</th>
<th>% O Cells (avg.)</th>
<th>Calc. Gr. O RBC Mass</th>
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<tbody>
<tr>
<td>Nov. 17, 1961</td>
<td>8.1</td>
<td>25</td>
<td>435 ml.</td>
<td>64</td>
<td>187 ml.</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>6.4</td>
<td>19</td>
<td>325 ml.</td>
<td>78</td>
<td>254 ml.</td>
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<tr>
<td>19</td>
<td>10.2</td>
<td></td>
<td></td>
<td></td>
<td>170-200 ml.</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.1</td>
<td>9.8</td>
<td>28</td>
<td>475 ml.</td>
<td>88</td>
<td>418 ml.</td>
</tr>
<tr>
<td>21</td>
<td>2.5</td>
<td>9.1</td>
<td>27</td>
<td></td>
<td>—</td>
<td>RBC's were transfused</td>
</tr>
<tr>
<td>22</td>
<td>3.5</td>
<td>9.1</td>
<td>27</td>
<td>460 ml.</td>
<td>88</td>
<td>400 ml.</td>
</tr>
<tr>
<td>23</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>7.9</td>
<td>9.8</td>
<td>30</td>
<td>510 ml.</td>
<td>100</td>
<td>510 ml.</td>
</tr>
</tbody>
</table>

kemia, dividing cells of the donor type persisted in the recipient's marrow. There was no evidence of "take" of donors cells other than those with the Ph1 chromosome when either marrow or peripheral blood was examined, and it was postulated that the Ph1 chromosome may provide a proliferative advantage.22 The donor in the present case did possess a Ph1 chromosome. The recipient chromosomes were not studied until after all AB cells had disappeared from the peripheral blood and, as expected, no Ph1 chromosome was demonstrated.

While the preceding considerations strongly favor a transplant of donor cells in the recipient, it is not clear which cells or cell types were involved. It is known from radiobiologic evidence that cells of the peripheral blood can repopulate the bone marrow and lymphoid organs.23,25 Moreover, the Ph1 chromosome probably involves a common precursor cell.26 These two pieces of evidence indicate the possibility that a common precursor served as the graft in this case. This is supported by the marked rise in platelets accompanied by some rise in white cells 2 weeks after transfusion. However, erythroid precursors alone conceivably could have proliferated since nucleated red cells were present in appreciable numbers in the donor's circulation at the time of transfusion.

In conclusion, it is postulated that erythroid precursors or undifferentiated multipotential stem cells contained in the blood of an AB donor with chronic myelogenous leukemia were successfully grafted to an O recipient whose immunologic mechanisms were temporarily impaired. The graft, which produced AB red cells and may or may not have produced platelets and white blood cells, was rejected 48 days after the first AB transfusion.

SUMMARY

A change in blood group was discovered in a group O boy with acute lymphocytic leukemia. Fifty-six per cent of his red cells were of group AB 6 weeks after he received white cell-rich plasma from a group AB donor with chronic myelogenous leukemia. It is concluded that hematopoietic cells in the peripheral blood of the donor survived and divided in the recipient, and were then rejected 6 weeks after transfusion. Factors favoring the acceptance of the graft, consideration of the type of cell or cells that proliferated, and the relation of this finding to recently published reports of survival of cells containing the Ph1 chromosome are discussed.
SUMMARIO IN INTERLINGUA

Un alteration del gruppamento sanguineo esseva discoperite in un puer de gruppo O con acute leucemia lymphocytic. Cinquanta-sex pro cento del erythrocytos del puer esseva gruppo AB sex septimanas post que ille habeva recipite plasma a alte contento leucocytic ab un donator de gruppo AB con chronic leucemia myelogene. Es concludite que cellulas hematopoietic in le sanguine peripheric del donator superviveva e se divideva in le recipiente e esseva rejicite sex septimanas post le transfusion. Es discutite factores que ha potite promover le acceptsation del graffo, le question del typo o typos de cellula que proliferava, e le relation inter iste constatation e recentemente publicate reportos de superviventia de cellulas que contine le chromosoma Ph1.

ACKNOWLEDGMENTS

The authors wish to thank Dr. E. Freireich and Dr. E. Frei, III for permission to study their patient and to include pertinent data from the clinical record. Thanks are expressed also to Dr. J. Whang and Dr. J. H. Tjio for performing the chromosome studies.

REFERENCES

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