Erythroid Homograft Following Leukocyte Transfusion in a Patient with Acute Leukemia

II. Serologic and Immunochemical Studies

By PAUL J. SCHMIDT, MITSUO YOKOYAMA, MARY H. McGINNIS AND ROBERT H. LEVIN

THE APPEARANCE of female Philadelphia (Ph') chromosomes in a male patient of blood group O who had received a transfusion of leukocyte-rich plasma from a female of blood group A with chronic myelogenous leukemia (CML), made it possible to conduct a study of blood groups during induction, growth and rejection of a hematopoietic graft. The recipient was being treated for acute lymphocytic leukemia. Observations were made on red cell, white cell and platelet antigens and their corresponding antibodies as well as the serum gamma globulin (Gm and Inv) groups. Only the data on red cell systems were conclusive. After the recipient showed doubling of his red cell mass, 85 per cent of these red cells were of the donor group A. Despite the presence of group A cells some free anti-A agglutinin was present throughout most of the two month graft life. Anti-B agglutinins appeared in very high titer. When the graft was rejected, immune anti-A hemolysin appeared and the cells of donor group A were rapidly destroyed.

MATERIALS AND METHODS

Transfusion

A detailed case report has been published in this issue. All data are chronicled from day 0, when the patient received 935 ml. of white cell-rich plasma from the group A female CML donor. Seven hundred and fifteen ml. of similar white cell material from a second donor, a male of blood group O with CML, were given on day 12. There is evidence from chromosome studies that this second transfusion did not result in a persistent graft and it will not be considered further in this report. In the terminal stage, after failure of the graft, 2 more transfusions of white cell-rich material were given, 670 ml. from the original group A CML donor and 200 ml. from another group O CML donor.

In addition to the white cell transfusions, 2 units* of group O red cells were transfused on day 0 and 2 units on day 13. Platelets from a total of 63 units of blood were given as concentrates or in platelet-rich plasma during the 87-day hospitalization.

All of these platelet products were from donors of blood group O with the exception

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*A unit is the product made from 500 ml. of whole blood.

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of one AB platelet concentrate (25 ml.) given 7 days before the first white cell transfusion, and 1200 ml. of platelet-rich plasma from 5 group A donors given on day 71. None of these transfusions would contribute enough red blood cells to influence the A grouping (<1 ml. red blood cells per platelet donor unit). During earlier admissions the recipient had received 94 units of blood products, all group O except for 4 platelet units of group A. These latter could have been the primary immunization dose of A antigen and the group AB platelet concentrate could have been the stimulus to anti-B formation as well.

Serologic Analysis

Anti-A and anti-B red cell agglutinin activity was graded according to the method of Race and Sanger. The observed values were corrected for the amount of anti-A and anti-B passively transferred in plasma. These data were calculated using the known volumes transfused, assuming an anti-A saline agglutinin titer of 1:200 and anti-B of 1:150 in the normal plasma and knowing the actual titers for the CML plasma. Since these agglutinins are mostly 19S gamma globulins, distribution in the intravascular component and a half-life of 5 days were assumed in these calculations.

Direct Coombs tests were done in parallel using 4 antiglobulin sera; a single reagent was used for quantitation. Antibody elution was performed by heating washed, saline-suspended red cells to 56 C. and rapidly removing the supernate.

Differential agglutination was done as previously described using avid anti-A from a single lot known to give a blank count of <1 per cent. The observed numbers of unagglutinated group O red cells per 100 total red cells were multiplied by the hemoglobin values. The results are expressed as grams of hemoglobin in O cells or A cells per 100 ml. whole blood.

The hemolysin tests were done using fresh human group A1 and group B red cells and, as a source of complement, normal human group O serum selected for its low content of anti-A and anti-B isoagglutinins and then absorbed at 0 C. with A1 and B red cells. Veronal buffer, pH 7.4, containing 0.15 M NaCl, 0.00015 M Ca++ and 0.0005 M Mg++, was used for dilutions and for the preparation of a 3 per cent cell suspension. Equal volumes of test reagent and cell suspensions were mixed and to this was added a double volume of the complement source. The tubes were incubated at 37 C. for 1 hour. Hemolysis was read visually and the titer was reported as the highest dilution of serum causing any hemolysis.

The platelets and white cells of the recipient six weeks after the initial leukocyte transfusions were typed in parallel with those of the donor. A battery of 20 typing reagents was used in a complement fixing system. The Gm and Inv gamma globulin groups of the donor and serial samples from the patient were determined in agglutination inhibition systems.

Immunofluorescence

Fractionation of serum was carried out by column chromatography using diethylaminoethyl cellulose (DEAE). The serum samples were dialyzed against 0.01 M sodium phosphate buffer, pH 8.1 and placed on a column equilibrated with the same buffer. The first or 7S gamma globulin peak was eluted using the same buffer as that used for dialysis. The final buffer, 0.2 M sodium phosphate, pH 8.1 containing 0.5 M NaCl, was then run through the column and the 19S gamma globulins obtained. These 7S and 19S fractions were concentrated at 4 C. to their original volume with negative pressure dialysis using a collodion membrane filter. The purity of the concentrated fractions was confirmed by immunoelctrophoresis and immunofiltration. The concentrated fractions were dialyzed against 0.01 M sodium phosphate buffer, pH 7.4 containing 0.15 M NaCl and these dialyzed samples were used for serologic study.

Immunofiltration was carried out for the measurement of immunoglobulin levels using a quantitative system (Immunoplates) according to the manufacturer’s directions. A
constant volume (.01 ml.) of each serum sample was placed in a well in an agar-gel impregnated with antiserum. Incubation was at 37 C. for 4 hours for measurement of 7S gamma globulin (Plate Lot No. 950U174) and at 22 C. for 16 hours for $\beta_2$A (Lot No. 950U175), and 19S (Lot No. 950U176) globulins. The diameters of the precipitin rings which resulted were measured under a "Finescale" magnifying lens.† The results were converted to mg. per cent by comparison with known standards.

**RESULTS**

The red cell groups of: (1) the recipient tested at a time when he had had no transfusions in seven months; (2) the CML donor; and (3) the recipient again on day 35 are given in table 1. There were only 3 systems in which the recipient initially lacked a factor present in the donor: A2, N, and K (Kell). The recipient became positive for all of these 3 factors. The growth of the A cell population so that it comprised 85 per cent of the red cell mass after the hematocrit had doubled is proof, independent of chromosome studies, of the establishment of a red cell chimera.

The results of our study are expressed as occurring during 3 phases: induction of the graft, its growth, and its rejection.

**Phase 1—Induction of the Graft**

- Day 0—CML transfusion, patient on antimetabolites.

*Hyland Laboratories, Los Angeles, Calif.
†Fine Scale Company, Los Angeles, Calif.
Table 2.—Levels of Free (Serum) and Bound Anti-A and Anti-B Antibodies

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ERYTHROID HOMOGRAFT AND ACUTE LEUKEMIA

Day 10—Marrow: leukemic cells, few megakaryocytes.

**Phase 2—Growth of the Graft**

Day 11—Platelets rising.

Day 18—Leukocytes rise, antimetabolites discontinued.

Day 19—Active erythropoiesis in marrow, 60 per cent of chromosomes XX Ph\(^1\) positive.

Day 24—Reticulocytes 2.5 per cent, 5 per cent of peripheral red cells of donor group A.

Day 33—Hemoglobin 7.8 Gm. per cent.

Day 41—Reticulocytes 16.3 per cent, 60 per cent of peripheral red cells of donor group A.

Day 49—Hemoglobin 14.2 Gm. per cent, 80 per cent of peripheral red cells of donor group A.

Day 51—Platelets below 150,000/mm.\(^3\)

**Phase 3—Rejection of the Graft**

Day 60—Antibody titers falling.

Day 61—Marrow still 90 per cent erythroid.

Day 65—Antimetabolite therapy reinstituted.

Day 67—Reticulocytes 2.4 per cent, hemoglobin falling.

These times are arbitrary since rates of cell maturation and loss were overlapping. A detailed discussion of these phases follows.

**Phase 1—Induction of the Graft**

**Peripheral Blood Cells:** The patient was given 4 group O red cell transfusions in this period. His hemoglobin level continued to fall at a rate of 0.3 Gm. per cent/day without any evidence for red cell production. At the time of take of the graft his red cell mass must have consisted almost entirely of a transfused O cell population. These O cells were of unknown N and Kell type and although this does not affect the measurement of the appearance of A cells, this does make it impossible to know whether or not the N and K factors were contributed by graft-produced cells.

**Antibodies:** In this inductive phase there was an initial high isoagglutinin titer. Then both agglutinins and hemolysins against B cells increased in quantity whereas anti-A agglutinin dropped. Anti-A hemolysin was not present. Direct Coombs tests were negative.

Immediately before the leukocyte transfusion from the A\(_2\) CML donor on day 0, the anti-A agglutinin titers were 1:128 by saline method and 1:256 by antiglobulin test against group A\(_1\) red cells, and 1:32 and 1:128, respectively, against group A\(_2\) cells. More antibody was passively transferred in 500 ml. of group O plasma on that day coincident with platelet and whole blood transfusions. On day 10, during the establishment of the graft and despite the injection of approximately 30 ml. of A\(_2\) red cells contained in the white cell transfusion on day 0, the titers were still 1:128 and 1:512 against group A\(_1\) cells and 1:32 and 1:256 against group A\(_2\) cells. The amount of passively transferred antibody was calculated and is expressed as the titer that would result in this patient (table 2). The remainder of the measured anti-A and
Table 3.—Titers of Isoagglutinins and Hemolysins in Serum Gamma Globulin Fractions

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Fig. 1.—Hemoglobin levels expressed as Gm. per cent total and Gm. per cent in group O and group A red cells. Serum isoagglutinin and hemolysin titers.

anti-B would be from active production and was calculated from the total observed titer. The active anti-A and anti-B values are plotted along with hemolysin titers and the results of differential agglutination in figure 1.

As shown in table 3, the 7S fraction of the serum sample on day 0 had neither anti-A nor anti-B agglutinin activity at 22 C. and 37 C. by the saline method. Only the antiglobulin test demonstrated this activity. Agglutinating antibody for both A and B were found in the 19S fraction. Hemolysin was not detected on that day. By day 10, anti-A and anti-B agglutinin activities were found both in the 7S and in the 19S fractions and anti-B hemolysin was present. (Although in normal group A and group B blood the isoagglutinin activity is generally in the 19S fraction and only appears in the 7S fraction after immunization, this is not true of group O sera. Some nonimmune group O sera contain agglutinin activity in the 7S as well as 19S fraction. The results of the immunochemical analysis on day 10 confirm the effect of passive transfer of large amounts of antibody in the transfused group O plasma.)

Serum Proteins: Just prior to day 0 transfusion which resulted in the graft,
the total protein was 6.8 Gm. per cent with 1 Gm. per cent of gamma globulin by paper strip electrophoretic analysis (our normal range of gamma globulin is 0.6 to 2 Gm. per cent). Immunodiffusion results are given in figure 2. A low 7S gamma globulin level of 420 mg. per cent was observed (normal values 600 to 1200 mg. per cent). The values of β2A and β2M were within our normal limits of 56 to 195 and 39 to 117 mg. per cent.

Beginning on day 0 the patient was started on intravenous gamma globulin therapy, receiving 18 ml. of the commercial 16 per cent solution of Immune Serum Globulin (Human) intravenously every day until day 32. This is shown in fig. 2 along with the dose of passively transferred gamma globulin calculated to have been in the transfused plasma.

The day 0 serum of the patient was Gm (a+b+c−x+) Inv (a+). The CML donor was Gm (a+b+c−x−) Inv (a+).

Phase 2—Growth of the Graft

Peripheral Blood Cells: The evidence for a red cell graft was clear. The per cent of circulating cells which were of the donor group A rose rapidly after day 24 to well over 80 per cent on day 53 when the hemoglobin went over 14 Gm. per cent. During this period the patient made 10 Gm. per cent of hemoglobin in 20 days and reticulocytes as high as 16.3 per cent were recorded. Over 90 per cent of the cells in the marrow were erythroid precursors and 100 per cent of cells in metaphase had the donor female Ph1 chromosome. Additional evidence that there was no erythropoiesis of the recipient's own O cells is seen in figure 1. The number of O cells shrank rapidly to levels expected to persist from the red cell transfusions of day 0 and day 13.

Platelet levels rose early and on day 26 peaked at over a million/mm.³ White cells never reached high levels and the maximum count was 6000/mm.³
on day 34. On day 45 the platelets and white cells of the recipient were typed in parallel with those of the first two CML donors. There were no differences in types between these samples which could be used to identify the genetic source of the recipient’s day 45 cells.

*Antibodies:* Free circulating anti-A agglutinins fell to low levels during this phase. These antibodies did not agglutinate the circulating A2 cells. Unexpectedly, anti-B antibody titers rose sharply and reached the abnormal level of over 2000 for agglutinin and 16 for hemolysin. This activity appeared first in the 19S fraction of gamma globulin and was later seen in the 7S fraction as well. Agglutination of A1 cells, a characteristic of most group O sera, was never seen.

Bound antibody soon appeared on the circulating cells (positive direct Coombs test) and when this material was eluted it agglutinated A but not B nor O cells on days 28 and 38. On day 49, and thereafter, group B cells were also agglutinated by the eluate. Neither the eluate nor the serum of any sample agglutinated O cells containing most of the other described red cell factors (Rh, Kell, Duffy, etc.).

*Serum Proteins:* The patient received daily injections of commercial Immune Serum Globulin (gamma globulin) from day 0 to 32. After 30 days of this therapy his circulating level of 7S gamma globulin was 50 per cent higher than the initial level without much change in $\beta_2$A and $\beta_2$M. By day 56 when most of the injected 7S material should have been cleared the values were again at day 0 level.

No changes in Gm or Inv types of the recipient were found.

**Phase 3—Rejection of the Graft**

*Peripheral Blood Cells:* The platelet count was 72,000/mm³ on day 60 but in another 10 days had fallen to 13,000. Granulocyte levels which had been as high as 80 per cent of 4300 cells/mm³ fell to 27 per cent of 2500 cells and a Pseudomonas septicemia appeared. Red cell production remained high for a longer period but when the hemoglobin began its rapid fall as shown in figure 1, the proportion of cells of group A fell from 85 per cent to 73 per cent indicating specific elimination of the graft-produced cells without loss of the O cells.

*Antibodies:* Although the anti-B agglutinin titer had been over 2000 on day 53, by day 60 it had fallen to 256 and by day 66 was 64. This was paralleled by complete disappearance of the anti-B hemolysin as well as the anti-A agglutinin. The 7S gamma globulin antibodies disappeared first. The direct Coombs test became weaker in this period.

The period of low antibody titers from day 60 to 66 was followed by a striking recrudescence of antibody. The rise in anti-A and anti-B agglutinins can be seen in table 2 to be more than expected from passive immunization by the transfusions which were begun again at this time. Hemolysin to B cells appeared again and for the first time anti-A hemolysin was seen as well. The titer of anti-A hemolysin (1:8) is evidence for active production rather than passive transfer.
Serum Proteins: Final conclusive evidence for a terminal immune response was provided by chemical quantitation of the immunoglobulins, the 7S gamma globulin level rising rapidly to above normal levels. Although Immune Serum Globulin injections were restarted on day 64, the 7S values rose from 420 mg. per cent to 1450 mg. per cent in 7 days whereas the earlier 30 days of therapy had succeeded only in raising the level from 420 mg. per cent to 680 mg. per cent. $\beta_2$M levels also rose although $\beta_2$A did not change.

**Discussion**

This is the second case reported from this laboratory of induction of a graft which produced circulating red cells following transfusion of peripheral blood from a patient with chronic myelogenous leukemia to a recipient with acute leukemia. The first case was observed only during rejection of the graft and the patient survived the episode. In the present case, induction of the graft was followed by a short clinical remission and loss of the graft immediately preceded the death of the recipient.

Although a tissue graft in humans has been described only a few times, enough animal experience has been obtained to expect a course of events which is the model for transplantation research: suppress the immune response, attempt transplant and then expect an immune response of graft versus host or host versus graft. In this successful, although unplanned, transplant of growing tissue there were two real differences from the anticipated model. In the first place the graft was induced in spite of at least a normal level of circulating antibody to the donor’s A red cell antigens. Secondly, during the active growth phase of the graft there was a continuing tremendous production of antibody to the related B antigen. These points will be discussed in detail.

Is antibody an aid rather than hindrance to graft induction?

The antibody titers shown for day 0 and day 7 in table 2 were obtained in the saline agglutination test. Titers of incomplete antibody (antiglobulin test) were 2 or 3 serial dilutions higher. Although much of the anti-A activity was present due to passive immunization, this should have been a truly hostile environment to A tissue. However, it should be noted that in the other cases of red cell graft that we have seen similar incompatibilities were present: an O recipient with anti-A and anti-B accepted hematopoietic tissue from an AB donor with anti-O and became more than half AB; an A recipient showed erythroid hyperplasia in his marrow with 99 per cent of the scoreable metaphases Ph positive after a transfusion of white cell-rich plasma from a group B, CML donor. It is possible that the red cell antibodies present during these tissue exchanges may actually have been stimuli to graft induction in the same way that phytohemagglutinin is a stimulus in tissue culture.

In the patient of Mathé et al. who accepted a graft of hematopoietic tissue from a pool of marrow and blood from six normal donors, the active tissue was from the donor who was most similar by histocompatibility tests and by study of the leukocyte antigens, but most dissimilar in red cell systems.
Although there was no pre-existing red cell incompatibility, this adds further
evidence of the limited importance of red cell antigens in tissue transplanta-
tion.10,11

Was the immune response during growth of the graft a function of the graft
or of the host tissue?12

During the growth of the A graft, free anti-A was at low levels. The surpris-
ing finding was the high level of antibody to B cells which appeared first as 19S
gamma globulin and later in the 7S fraction as well. This immune response
began while the patient was receiving antimetabolite therapy which was only
discontinued on day 18. This antibody could have been: (1) an anti-B made
by immunologically competent cells of the A donor line responding to the
stimulus of the group AB blood product given on day 7; (2) an anti-B made
by O recipient tissue responding to the same stimulus; or (3) a “cross-reacting”
anti-A+B made by the O recipient in response to the A stimulus of the graft.

We are of the opinion that the antibody was anti-B because (1) the free
anti-B titer was already over 2000 at a time when anti-B was not yet elutable
from the antibody coated red cells as it should have been if this were the
“cross-reacting” antibody of immune O serum; and (2) the free anti-B fell
rapidly in the day 60-70 period without evidence that this was because “cross-
reacting” anti-A+B was binding to donor line A tissue, i.e., the direct Coombs
test became weaker rather than stronger and the production of A cells
continued as shown by marrow erythropoiesis and the continued presence of
reticulocytes until day 67. The fall in antibody titer preceded reinstitution of
antimetabolite therapy on day 65.

In addition to anti-B some “cross-reacting” anti-A + B was being made
by O tissue since it continued to be possible to elute anti-B activity from the
circulating cells after the anti-B titers had fallen.

We had hoped that serum (gamma globulin) groups would enable us to
distinguish whether the immune anti-B was made by donor or recipient
tissue. Unfortunately there were no dissimilarities which permitted unequiv-
ocal distinction.

It is also unfortunate that the types of the leukocytes and platelets in the
donor and the grafted recipient (day 45) were such that it was not possible
to identify the properties of each line in the circulating population. The
complement fixation technic used had proved very useful in other studies in
the identification of minor cell populations but in this case pregraft recipient
cells were not available for test.

Final Immune Response

The death of the graft was followed by clear evidence that this was an
immmunologic death. The final antibody rise in the terminal phase was in both
anti-A and anti-B antibodies which could only have been produced by the
patient’s own tissue. Anti-A hemolysin appeared for the first time. However,
these responses in the ABO red cell system may be of no direct significance
but only reflect an overall rise in antibody, including antibodies to crucial
tissue antigens. They were not accompanied by appearance of specific antibody in the Kell system which is usually quite antigenic in transfusion practice. The striking rise in serum 7S gamma globulin levels at this time was the key indication of a general immune response.

**Summary and Conclusions**

The establishment of a hematopoietic graft of stem cells from a donor with chronic myelogenous leukemia in a patient with acute leukemia took place in the face of ABO red cell group incompatibility. The donor was group A and the recipient who was group O gradually increased his red cell mass to become 80 per cent group A.

There was both active and passive immunity to A present at the time of induction of the graft. The graft flourished despite persistent anti-A agglutinins and an immune response in the B agglutinin and hemolysin system.

Failure of the graft coincided with a fall in antibody levels and was followed by a second immune response which included marked elevation of 7S gamma globulin levels. Red cell incompatibility was not a barrier to this graft and failure of the graft was probably due to other immune mechanisms.

**Summario in Italian**

Le establimento de un graffo hematopoietic de cellulas primordial ab un donator con chronic leucemia myelogene in un patiente con leucemia acute occurreva in le presentia de incompatibilitate erythrocytic de systema ABO. Le donator pertineva al gruppo A, e le recipiente, pertinente al gruppo O, gradualmente augmentava su massa erythrocytic usque 80 pro cento de illo pertineva al gruppo A.

Al tempore del induction del graffo, immunitate contra A, tanto active como etiam passive, esseva presente. Le graffo prosperava in despecto de persistente agglutininas anti A e un responsa immunologic in le systema de agglutinina B e de hemolysina.

Le final disfallimento del graffo coincideva con un declino del nivello de anticorpore, lo que esseva sequite de un secunde responsa immunologic que includeva un marcate elevation del nivellos de globulina gamma 7S. Incompatibilitate erythrocytic non esseva un obstaculo pro le graffo e le disfallimento de isto esseva probablemente causate per altere mechanismos immunologic.

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**References**


ERIYTHROID HOMOGRAFT AND ACUTE LEUKEMIA

Erythroid Homograft Following Leukocyte Transfusion in a Patient with Acute Leukemia: II. Serologic and Immunochemical Studies

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