Therapy of Acute Leukemia by Whole-Body Irradiation and Bone Marrow Transplantation from an Identical Normal Twin

By John B. Atkinson, Francis J. Mahoney, Irving R. Schwartz and Joseph A. Hesch

Experimental efforts directed to the better control of leukemia have recently taken a new direction basically different from present therapeutic approaches. This work stems from the modifications of acute irradiation injury effected by the transplantation of living hematopoietic cells. Experimentation in the therapy of murine leukemia with lethal irradiation followed by isologous, homologous and heterologous bone marrow transplantation with resultant survival of the irradiated animals has been reported. A paper recording preliminary findings on homologous bone marrow transplantation in humans with indication of success has recently been published. In animal studies, the most serious drawback has been the development of a late immunologic reaction with death of the animals when other than isologous marrow was transplanted. The purpose of this report is to record the results in a human case of acute leukemia which was treated by total body irradiation followed by bone marrow transplantation from a normal identical twin with indication of a successful isologous transplantation.

Case History

A female child, age 18 months, was admitted to the Thomas M. Fitzgerald Mercy Hospital with a history of anorexia, tiredness and pallor of five weeks' duration. The patient had had a normal delivery and birth. The aforementioned symptoms were of increasing severity, particularly during the two weeks prior to admission. The attending pediatrician examined the patient and studies revealed a marked anemia. Past medical history and familial history were noncontributory. Except as mentioned in the history of the patient's illness, systemic review revealed no abnormal findings. Dietary history revealed an adequate food intake, including supplemental vitamin therapy.

Physical examination revealed a temperature of 101 F., a pulse of 120 and regular, and respirations of 34 per minute. The patient's weight was 25 pounds. There was marked pallor evident, slight gingival hyperplasia, slight erythema of the nasal and oral pharyngeal mucosa, bilateral posterior cervical and anterior cervical lymphadenopathy with a maximal node size reaching approximately 2 cm. in diameter, bilateral axillary lymphadenopathy with a maximal node size of approximately 1 cm. in diameter in the left axilla, and no discernible hepatosplenomegaly or other significant lymphadenopathy. Laboratory studies revealed a hemoglobin of 4.0 Gm. (26 per cent), a red blood cell...
count of 1,430,000 with a hematocrit of 13 volumes per cent, and a white blood cell count of 10,650. The differential count was reported as 6 per cent segmented neutrophiles, 8 per cent neutrophiles nonsegmented, 5 per cent metamyelocytes and 81 per cent lymphocytes seen with some question as to the maturity of some of the cells. Reticulocyte count was 5 per cent. Platelets were 40,000 per cu.mm. Examination of the smears of the peripheral blood revealed 3 nucleated red blood cells per hundred leukocytes. The red cells appeared hypochromic and microcytic with some anisocytosis, poikilocytosis and basophilia. Additional smears of the peripheral blood revealed approximately 40 per cent lymphoblasts. The bone marrow aspiration biopsy revealed 98 per cent lymphoblasts. A diagnosis of acute lymphoblastic leukemia was established on the basis of the findings in the peripheral blood when reviewed coupled with the bone marrow aspiration biopsy. X-rays of all bones revealed normal findings for the child’s age. Chest x-ray showed minimal exaggeration of hilar shadows bilaterally and accentuation of bronchovascular markings to both bases. Intravenous urogram was normal.

The patient’s general condition was poor with no evidence of improvement on supportive therapy prior to the establishment of the diagnosis. Preliminary therapy with prednisone was initiated, 5 mg. four times daily. The patient was transfused with 750 cc. of blood in 250 cc. increments with an elevation of hemoglobin to 8.5 gm. per cent, red blood cell count of 3,040,000 and the hematocrit to 30 volumes per cent. During an interval of 20 days the white blood cell count remained between 9,000 and 10,000, with a decrease in the number of lymphoblasts from 40 per cent to 9 per cent. Platelets remained approximately stationary at 40,000 per cu.mm. The patient had a twin sister who was found to be normal on examination. The whole family was typed with regard to: ABO, CDE and MN group (table 1). The twins were found to have exactly the same types, as indicated in the table. In addition, both were Dii negative, and compatibility tests on both major and minor side by saline, serum-albumin and antiglobulin methods showed no reaction.*

Reliable obstetrical reports were not available with regard to placental membrane. Close study and photographs (fig. 1) indicated a marked similarity between the two children which, coupled with the findings regarding their various blood types, were considered to indicate the probability of the twins being identical. Accordingly, a consultation was requested regarding the possibility of using radiotherapy followed by a bone marrow transplantation from the identical twin. It was the general thought of the consulting hematologist, radiotherapist and attending pediatrician that this might offer more than the routine methods of therapy.

On July 10, 1957, total body irradiation was given to the patient. A 200 cm. TSD was used with an 80 by 80 cm. field. She was centered in this field in a prone “frog leg” position. The length of the patient was 57 cm. and thus the peripheral “drop off” of irradiation was less than 4 per cent. With added filtration of 0.5 mm. Cu plus 1 mm. of Al, 220 KVCP was used. Bolus was used around the body and extremities. In air, 300 roentgens was given as a single dose. The greatest diameter of the child was 10 cm. and the dose given to the patient as measured on a phantom was:

---

* Appreciation is expressed to Herbert S. Bowman, M. D., of the Harrisburg Hospital, for the complete typing of this family.
FIG. 1.—Five weeks post-transplantation. Normal twin, M. McD., on left; leukemic twin, L. McD., on right, showing slight facial changes due to corticoid and corticotrophin therapy.

Skin 380 roentgens
5 cm. below skin 255 roentgens
Exit dose 165 roentgens
Dose rate 4.5 air roentgens per minute

The child became quite lethargic near the end of treatment. Nausea and vomiting commenced within 15 minutes after completion. Nausea, vomiting and elevated temperature continued during the following 17 hours preceding the transplantation of bone marrow.

The next morning, under general anesthesia, bone marrow was obtained from the normal twin. A total of 31 bone marrow aspirations was done (see table 2) with an average volume of 1.3 cm. and a total volume of 40.3 cm. Each bone marrow aspirate was withdrawn into siliconized syringes containing 1.0 cc. of an anticoagulant (0.1 per cent disodium versenate in phosphate buffer saline with a pH of 5.5) and immediately and separately given to the leukemic twin through a polyethylene canulated anterior tibial vein. Number 16 marrow needles were used. Random cell counts of the nucleated elements revealed an average of 65,800 per cu.mm. of blood marrow aspirate, giving an estimated total number of nucleated cells of $2.65 \times 10^8$. This average approximated $234 \times 10^6$ nucleated cells per Kg. of body weight for the recipient. Because of the thrombocytopenia (40,000 per cu.mm.), immediately following the transplantation, the patient was given 70 cc. of
THERAPY OF ACUTE LEUKEMIA

TABLE 2.—Bone Marrow Aspirations from Normal Twin

<table>
<thead>
<tr>
<th>Number of Aspirations</th>
<th>Average Volume of Each Aspiration</th>
<th>Estimated Total Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Tibia</td>
<td>4</td>
<td>1.3 cc.</td>
</tr>
<tr>
<td>Right Iliac</td>
<td>14</td>
<td>40 cc.</td>
</tr>
<tr>
<td>Left Iliac</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Random Cell Counts of Nucleated Elements</th>
<th>(After 1:1 dilution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Tibia</td>
<td>32,650 (Early Aspiration)</td>
</tr>
<tr>
<td>Right Iliac</td>
<td>64,000 (Early Aspiration)</td>
</tr>
<tr>
<td>Left Iliac</td>
<td>25,000 (Early Aspiration)</td>
</tr>
<tr>
<td>Left Iliac</td>
<td>10,000 (Last Aspiration)</td>
</tr>
<tr>
<td>Average</td>
<td>32,900 (With 1:1 dilution)</td>
</tr>
<tr>
<td>Average</td>
<td>65,800 (Corrected for versenate dilution)</td>
</tr>
</tbody>
</table>

Estimated Total Number Nucleated Cells: 2,651,740,000 (2.65 x 10^9)

Whole fresh blood taken directly from a heparinized donor who had previously been typed, cross-matched and had a nonreactive V.D.R.L. test. Following this, the patient received no further blood transfusion during the course of the hospitalization.

After the transplantation the patient became afebrile within 24 hours. The only complication was the development of very fine, transient tremors of the extremities which lasted approximately two to three seconds and which occurred every two to three minutes. There were no generalized convulsive symptoms. Signs and symptoms of hypocalcemia were absent. The tremors disappeared when the patient was awake. They were absent after the first 24 hours. Parenteral fluids were given continuously for three days. Prednisone therapy was continued but was gradually decreased until three days prior to discharge when it was completely discontinued. Zinc corticotrophin, 20 units, was given by intermittent intramuscular injection every third day. Following the transplantation combined penicillin and streptomycin were administered for 10 days.

After the transplantation appetite improved markedly and fluid intake was adequate. The patient was very active and ambulatory on the pediatric floor within one week of the irradiation and transplantation. The subsequent course was essentially uneventful until 18 days after the irradiation when the patient developed an upper respiratory infection with an elevation of temperature to 102 F. which, coincidental with or in response to tetracycline therapy, returned to normal in 24 hours. Hair loss began on approximately the 17th day after irradiation. It receded a maximum in 24 days. The estimated loss was approximately 20 to 30 per cent.

Bone marrow study was carried out one week following the transplantation and revealed sparse cellularity. An occasional megakaryocyte, however, could be found on extensive searching. The myeloid to erythroid ratio was 3 to 1. No "blast" cells could be found in the aspirate nor could any be found in the peripheral blood. A second bone marrow aspiration biopsy, two weeks after the transplantation, showed a myeloid to erythroid ratio of 1 to 1.2. Cellularity was fairly good and megakaryocytes were adequate. Nucleated cell count was 18,000 with a peripheral count of 3,000. A third bone marrow biopsy, four weeks after the transplantation, demonstrated a myeloid to erythroid ratio of 1 to 1, with normal maturation in both series. No stem cell or lymphoblast could be found in the marrow aspirate specimen nor could any be found in the peripheral blood smears. The nucleated cell count on the marrow at that time was 30,000, with a peripheral count of 3,000. The patient's hemoglobin remained essentially stable between 8.5 Gm. and 9 Gm. per hundred cc. of blood. The patient was discharged from the hospital five weeks following the transplantation. At home she did fairly well with an excellent appetite and normal interest and activity.

Seven weeks subsequent to the transplantation the patient began to develop anorexia, listlessness, pallor, and was found to be febrile. Eight weeks after the transplantation, examination of smears from the peripheral blood and the bone marrow revealed a relapse.
into an acute lymphoblastic picture. The patient was hospitalized and transfused. Prednisone therapy was initiated. An excellent response was obtained within two weeks and therapy was continued (fig. 2).

Four weeks later, refractoriness to corticoid therapy was manifest and 6-mercaptopurine therapy was initiated with good results. Seven weeks later the patient again entered an acute blastic phase and hospitalization was required for transfusion. Amethopterin therapy was started. Over-all response was not satisfactory. Re-admission to the hospital was required one month later for transfusion and supportive care, and the child died with the manifestations of an acute leukemia refractory to all modalities of therapy eight months after the initial irradiation and marrow transplantation attempt. The normal twin who served as donor has remained well to the present without evidence of leukemia or sequelae or complications from the multiple aspirations which were carried out one year ago.

**DISCUSSION**

Experience in this case is believed to indicate, as suggested by others, the possibility of inducing a remission in acute leukemia when irradiation therapy followed by marrow transplantation is used. The absence of signs and symptoms of irradiation sickness is believed to indicate a protective influence of isologous bone marrow transplantation when such therapy is used in humans, which is in keeping with the results found in animal experimentation. The average dose of irradiation delivered to this child was 255 tissue roentgens. This amount of irradiation is well below the minimal lethal dose for a normal human.

In future work it would probably be advisable to consider a total body
irradiation dose of 400 to 600 roentgens followed by the attempted bone marrow transplantation. Furthermore, recent animal work has indicated that a homologous marrow transfer from a different strain of mice is more effective in suppressing the leukemic process than a similar transplant of isologous marrow from the same strain of mice. However, the late immune reaction was responsible for the death of those animals surviving the leukemia. This suggests that isologous transplants in humans may be less desirable than homologous transplants and indicates that the principle difficulty to be overcome may be the development of the homologous reaction. The work in primates is somewhat encouraging in this regard, insofar as the homologous monkey experiments simulate the human problem—that of treating a mongrel population rather than pure strains. In pure strain rodents, homologous reaction occurs quite regularly with great severity. However, in the mongrel primate group homologous reactions were less frequent and less marked. In humans the use of near relatives as donors may materially increase the degree of acceptability of homografts following irradiation. Since so few have isologous siblings, the plausibility of using isologous transplants will necessarily be limited; and where feasible more than 255 tissue roentgens would be required as irradiation preliminary to transplantation.

CONCLUSIONS

1. In this case of acute leukemia, irradiation of 255 r quickly eliminated detectable “blastic” elements in both the peripheral blood and the bone marrow, as indicated by changes in marrow and peripheral blood.

2. This patient received 255 tissue roentgens. Followed by isologous transplantation this dose was well tolerated. In the future larger doses should be considered. In this patient the dose of irradiation given is believed to have been adequate to destroy all leukemic cells or prevent their recurrence.

3. In this case isologous marrow from an identical twin was used. No information was gained about immune response. Animal experimentation would indicate that possibly a better result regarding control of the leukemia may be obtained from the use of homologous marrow.

4. Twenty per cent to 30 per cent temporary depilation occurred within 21 days after the irradiation in this case. There were no changes in the skin or mucous membranes. Except for vomiting during the first 17 hours following the irradiation, there were no gastrointestinal symptoms. There were no changes in the lenses or corneae of the eyes. While the level of irreparable damage to vital organs may be a sharp and critical amount, it would appear to be well above 255 tissue roentgens, and higher doses should probably be used in the future with protection possibly afforded by marrow transplantation.

SUMMARIO IN INTERLINGUA

Es presentate le resultatos obtenite in le caso de un puera de 18 menses de etate, suffrente de leucemia acute e tractate per irradiation del corpore integre e subsequentemente transplantation de medulla ossee ab un normal gemina identic.
2. Le irradiazione di 255 r eliminava rapidamente omne detegibile elementi “blastic” tanto in le sanguine peripheric como etiam in le medulla ossee.

3. Le irradiazione per 255 r al histos, sequite per transplantation isologe, esseva ben tolerate. In le futuro, plus grande doses deberea esser prendite in consideration. Es opinate que in le patiense del presente reporto, le dose de irradiazion usate esseva adequate pro destruer omne le cellulas leucemic o pro prevenir lor recurrentia.

4. Nulle information esseva colligite con respecto al responsa immunologic evocate per le transplantation de medulla ossee isologe ab un gemina identic. Experimentos in animales pare indicar que un melior resultato, relative al domination del leucemia, es possibilemente obtenibile per le uso de medulla homologe.

4. Occurreva, intra 21 dies post le irradiazion, un depilation temporari amontante a inter 20 e 30 pro cento. Nulle alterationes esseva presente in le pelle e le membranas mucose. A parte le occurrentia de vomito durante le prime 17 horas post le irradiazion, nulle symptomas gastro-intestinal esseva notate. Nulle alterationes esseva trovate in le lentes o le corneas del oculos. Le nivello critic a que le irradiazion resulta in irreparabile injurias de organos vital es probablemente un magnitude concise, sed illo pare esser considerablemente supra le nivello de 255 r al histos, e il es probable que in le futuro plus grande doses debe esser usate, possibilemente con le provision de un efecto protectori per le transplantation de medulla.

REFERENCES


Therapy of Acute Leukemia by Whole-Body Irradiation and Bone Marrow Transplantation from an Identical Normal Twin

JOHN B. ATKINSON, FRANCIS J. MAHONEY, IRVING R. SCHWARTZ and JOSEPH A. HESCH