Successful Allogenic Bone Marrow Transplantation in Man: Chimerism, Induced Specific Tolerance and Possible Anti-Leukemic Effects


It has been demonstrated in various animal species that allogenic (homologous) bone marrow transplantation is possible after conditioning the recipient by a lethal dose of total-body irradiation.19 A successful transplant is usually complicated by a secondary syndrome,2 the mechanism of which probably involves the reaction of immunologically competent cells against host antigens.4

If the recipient is leukemic, the immune reaction of the graft against the leukemic cells3,20,25,28,29 and perhaps against the leukemia virus19 may prove to be a powerful therapeutic weapon. Hence, the idea of using allogenic bone marrow grafts in the treatment of leukemia. The object in such cases would be to obtain prolonged acceptance of the graft, followed by the secondary syndrome which indicates the graft versus host reaction, and then to control the undesirable effects of the secondary syndrome.

Case Report

Before Irradiation

B. B., a 26-year-old physician, had been suffering from acute lymphoblastic leukemia since August, 1961. The first progressive phase was treated with Δ-1-cortisone 100 mg. daily from September 10 to November 8, 1961. An apparently complete remission was obtained during the course of which (from November, 1961 to December, 1962) the patient received 6-mercaptopurine in doses of between 50 and 200 mg./day as limited by his gastrointestinal and hematologic tolerance. On December 10, 1962, a differential count of the bone marrow revealed 24 lymphoblasts per 100 nucleated cells. A course of Leurocristine (1 injection of 3 mg./week for 4 weeks) was both ineffective and poorly tolerated from gastrointestinal and neurologic standpoints. On January 7, 1963, the percentage of lymphoblasts in the marrow had risen to 33. Another course of Δ-1-cortisone was instituted and continued until March 18th. This reduced the lymphoblasts in the bone marrow to 11 per cent. It was decided to attempt allogenic bone marrow transplantation after total-body irradiation; further relapse was noted while preparations for this procedure were being made.

On April 17, 1963, blood studies showed: hemoglobin 16 Gm./100 ml., red blood cells 4,900,000/cu. mm., white blood cells 3,300/cu. mm., with 48 neutrophils and 19 lymphoblasts, and platelets 210,000/cu. mm. On the same day the bone marrow was found to

From the Department of Hematology, Institut Gustave-Roussy, and Institute Cancérologie et d’ Immunogénétique, Villejuiff, Seine.

Study carried out with the aid of the Centre for Nuclear Studies, Fontenay-aux-Roses (Contract no. 5.262/r) and the National Institutes of Health, Bethesda, Md. (Contract no. CA 05-703-03).

Submitted Nov. 21, 1963; accepted for publication May 20, 1964.

179

Blood. Vol. 25, No. 2 (February), 1965
contain 55 lymphoblasts, 19 granulocytes and 10 erythroblasts per 100 cells. The patient had never received a blood transfusion at any time.

**Total-Body Irradiation and its Immediate Consequences**

Before irradiation, 300 mg. daily of methyl-nitro-imidazolyl-mercaptopurine ("Imuran," Burroughs-Wellcome) was given for 4 days. Irradiation was then carried out in two sessions, on April 17th and 18th at the Gustave-Roussy Institute in an installation consisting of two Cobalt-60 sources, the distance between the patient and each of these sources being 400 cm. The dose was homogenous in all directions in the center of a volume 200 x 100 x 100 cm. Scatter was not greater than 5 per cent and only the legs were exposed to an overdosage of about 15 per cent. A dose of 3.76 r./minute was delivered to the center of the body. Total time of exposure was 168 minutes, for a total dose of 800 r. The irradiation was well tolerated, but vomiting occurred soon afterward with progressive anorexia. The hematologic consequences were usual for such radiation and are shown in figures 1 and 2. Myeloid cells were no longer found in the marrow after April 22nd.

The patient was kept under condition of maximum asepsis. Before irradiation, we had assured ourselves that he harbored no pathogenic organisms. His hospital room was kept aseptic with pressurized air, ultra-violet rays and his food was sterilized. Physicians and nurses were checked bacteriologically and observed the precautions usually taken in aseptic rooms. During the first 10 days, the myelo-lymphoid aplasia was perfectly well tolerated.

Six bone marrow donors were selected, all closely related to the patient, viz., his father (H), three brothers (D, M, P), mother (G) and sister (F). Table 1 shows the erythrocyte (Dr. C. Salmon) and serum (Dr. C. Ropartz) phenotypes and table 2 the results of a study of leukocyte antigens (Dr. J. Dausset) of the donors and the recipient.

Histocompatibility between the recipient and the various donors was studied by a test previously described by us. A subject was immunized by a skin graft from the patient and then given a skin graft from each of the intended bone marrow donors; elimination of these grafts was as follows: H and P, on the 12th day, M and F, the 14th day; D, the 15th day; and G, the 22nd day.

**Transfusion of Bone Marrow**

On April 23rd, the patient was given an intravenous infusion of 2000 ml. of bone marrow mixture obtained from the six donors (about 330 ml. per donor). This volume represented 5.8 x 10^10 nucleated cells (H. B., 8.27 x 10^9 cells; D. B., 9 x 10^9; M. B., 9.9 x 10^9; P. B., 14.4 x 10^9; C. B., 7.9 x 10^9; F. B., 9.2 x 10^9). During the marrow infusion, which was completed in 5 hours, 1700 ml. of blood was removed. The entire procedure was well tolerated.

**Myeloid Restoration and the Secondary Syndrome**

After the bone marrow infusion, the patient received a daily transfusion of 3.5 x 10^11 irradiated platelets because of thrombocytopenia. He was also given 30 Gm. of epsilon-amino-caproic acid daily, both as an antihemorrhagic and as an inhibitor of immune reactions; in addition, he received mycostatin (4 million units per day) after gingival mycosis had been observed.

Reticulocytes were seen on April 25th and disappeared again on April 29th and 30th. They reappeared on May 2nd and at that time a few hematopoietic cells (erythroblasts, promyelocytes and myelocytes, megakaryocytes) were noted in bone marrow smears. Marrow restoration progressed slowly; the level of polymorphonuclear neutrophils did not rise until after May 8th and the platelets rose above the count of 50,000/cu. mm. only after May 20th.

From the first days of May, marked diarrhea and vomiting occurred, soon followed by an eruption consisting of small erythematous, rubuliform spots on the back and thighs, which became generalized by May 8th (fig. 3). The lesions became confluent and assumed a violet pruriginous appearance, and the skin was slightly edematous. The temperature was
about 39 C. Despite negative blood cultures, propionylethromycin, 1.25 Gr./day, was given. Fine desquamation of the skin began about May 15th (fig. 4). Diarrhea and vomiting continued, severe myalgias occurred and the patient lost 15 Kg. During the next 10 days, the vomiting diminished in frequency and the diarrhea improved. The eruption was still intense and continued to desquamate. A skin biopsy showed considerable acanthosis. There were many dispersed eosinophilic epithelial cells with pyknotic nuclei in the basal and superficial layers of the epithelium; some vacuolized basal cells were also seen. An
Table 1.—*Erythrocyte and Serum Phenotypes of the Host and Donors*

<table>
<thead>
<tr>
<th>Host</th>
<th>ABO</th>
<th>Mn/Sa</th>
<th>P</th>
<th>CDE</th>
<th>K</th>
<th>Kp</th>
<th>Le</th>
<th>Fy</th>
<th>Jk</th>
<th>Gm</th>
<th>Inv</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. B.</td>
<td>O</td>
<td>N/S</td>
<td>P₁</td>
<td>CeDec</td>
<td>K₁</td>
<td>(a⁻)</td>
<td>a⁻b⁺</td>
<td>a⁺</td>
<td>a⁺</td>
<td>a⁻b⁺x⁻e⁺</td>
<td>1⁺a+b⁺</td>
</tr>
<tr>
<td>Donors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. B.</td>
<td>O</td>
<td>N/S</td>
<td>P₁</td>
<td>CeDec</td>
<td>K⁻</td>
<td>(a⁻)</td>
<td>a⁻b⁺</td>
<td>a⁺</td>
<td>a⁺</td>
<td>a⁻b⁺x⁻e⁺</td>
<td>1⁻a⁻b⁺</td>
</tr>
<tr>
<td>D. B.</td>
<td>O</td>
<td>N/S</td>
<td>P₁</td>
<td>CeDec</td>
<td>K⁻</td>
<td>(a⁻)</td>
<td>a⁻b⁺</td>
<td>a⁺</td>
<td>a⁻</td>
<td>a⁻b⁺x⁻e⁺</td>
<td>1⁻a⁻b⁺</td>
</tr>
<tr>
<td>M. B.</td>
<td>O</td>
<td>N/S</td>
<td>P₁</td>
<td>CeDec</td>
<td>K⁻</td>
<td>(a⁻)</td>
<td>a⁺b⁺</td>
<td>a⁺</td>
<td>a⁻</td>
<td>a⁻b⁺x⁻e⁺</td>
<td>1⁻a⁻b⁺</td>
</tr>
<tr>
<td>P. B.</td>
<td>O</td>
<td>N/ss</td>
<td>P₁</td>
<td>CCDec</td>
<td>K⁻</td>
<td>(a⁻)</td>
<td>a⁺b⁺</td>
<td>a⁺</td>
<td>a⁺</td>
<td>a⁻b⁺x⁻e⁺</td>
<td>1⁻a⁻b⁺</td>
</tr>
<tr>
<td>G. B.</td>
<td>O</td>
<td>N/S</td>
<td>P₁</td>
<td>CCDec</td>
<td>K⁻</td>
<td>(a⁻)</td>
<td>a⁻b⁺</td>
<td>a⁻</td>
<td>a⁻</td>
<td>a⁻b⁺x⁻e⁺</td>
<td>1⁻a⁻b⁺</td>
</tr>
<tr>
<td>F. B.</td>
<td>O</td>
<td>N/S</td>
<td>P₂</td>
<td>CCDec</td>
<td>K⁻</td>
<td>(a⁻)</td>
<td>a⁻b⁺</td>
<td>a⁺</td>
<td>a⁺</td>
<td>a⁻b⁺x⁻e⁺</td>
<td>1⁻a⁻b⁺</td>
</tr>
</tbody>
</table>
Table 2

<table>
<thead>
<tr>
<th>Host Donor</th>
<th>+</th>
<th>-</th>
<th>-</th>
<th>+</th>
<th>Number of Similar Reactions</th>
<th>Number of Different Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host &amp; H. B.</td>
<td>9</td>
<td>26</td>
<td>4</td>
<td>9</td>
<td>35</td>
<td>13</td>
</tr>
<tr>
<td>Host &amp; D. B.</td>
<td>6</td>
<td>25</td>
<td>7</td>
<td>10</td>
<td>31</td>
<td>17</td>
</tr>
<tr>
<td>Host &amp; M. B.</td>
<td>7</td>
<td>20</td>
<td>6</td>
<td>15</td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td>Host &amp; P. B.</td>
<td>10</td>
<td>28</td>
<td>3</td>
<td>7</td>
<td>38</td>
<td>10</td>
</tr>
<tr>
<td>Host &amp; G. B.</td>
<td>6</td>
<td>27</td>
<td>7</td>
<td>8</td>
<td>33</td>
<td>15</td>
</tr>
<tr>
<td>Host &amp; F. B.</td>
<td>11</td>
<td>28</td>
<td>2</td>
<td>9</td>
<td>37</td>
<td>11</td>
</tr>
</tbody>
</table>

*+ = agglutination; - = no agglutination.*

infiltrate of mononuclear cells surrounded the adnexa and small vessels in the dermis; a few of these cells appeared to be invading the epithelium (fig. 5).

By May 25th, peripheral leukocyte and platelet counts were indicative of good marrow restoration. Hyperbasophilic cells were now noted in the blood (2-8 per cent) and bone marrow (3-11 per cent); in addition, progressively increasing eosinophilia, up to 55 per cent (on May 30th) appeared (fig. 2).

On May 28th, desquamation was generalized and the myalgia had exacerbated. The diarrhea and nausea persisted but was less severe. The temperature fluctuated around 38°C.

Mild cervical polyadenopathy occurred by June 1st associated with monocytes in the blood. A cervical lymph node removed for examination showed histologically extreme lymphocytic aplasia with complete absence of lymphoid follicles (fig. 6). The reticuloendothelial structure was normal and the relative number of nuclear histiocytes was increased (fig. 7). Few plasmocytes were seen but there were many granulocytes and eosinophils. Stained smears of the lymph node revealed a distinct predominance of histiocytes over lymphocytes; there were numerous hyperbasophilic cells, mostly histiocytes, a few plasmocytes, numerous eosinophilic granulocytes and no lymphoblasts (fig. 8). Electron-microscopic examination confirmed that the cellular population chiefly consisted of mobile histiocytes rich in ribosomes but with no ergastoplasmic membranes. Lymphocytes were rare and plasmocytes few.

Electrophoresis of the plasma proteins revealed diminished gamma globulin values (6-7 Gm./L.); immunoelectrophoresis showed diminution of gamma, beta 2A and beta 2M globulins with increased beta 1A globulins and alpha 2 macroglobulin values. The direct and indirect Coombs tests were negative.

The polyadenopathy remained palpable for about 8 days. By June 7th the eruption was replaced by progressively intensifying pigmentation. Hepatomegaly was apparent and the liver edge was felt 3 cm. below the costal margin. Liver involvement had been suspected for some time because of the elevated values of the serum enzymes. The SGOT level was three to four times as high as the SGPT, the inverse of that in virus hepatitis. The increase in aldolase, however, was of the same order as that usually seen in hepatitis. The increase in OCT indicated that hepatic involvement existed because this enzyme is found only in the liver. Because of the hepatic dysfunction Δ-1-cortisone was begun in a dosage of 10 mg. daily and increased to 25 mg. daily on June 19th. The drug had no effect on the temperature which remained at about 38°C and caused no notable regression of the hepatomegaly. Its administration, however, was followed by a decrease in eosinophils.

Because of a slight cough, an x-ray of the chest was taken on June 15th which revealed diffuse miliary lesions. No Koch's bacilli were found in the sputum; nevertheless, the lesions were considered to be of tuberculous origin and were treated as such. INH (900 mg. daily) and streptomycin (1 Gm. daily) were started and propionyl-erythromycin was discontinued. The patient was slightly dyspneic for 10 days after which the respirations became normal.

*Lactic dehydrogenase 600; glutamic-oxalacetic transaminase (SGOT) 92; ornithine carbamyl transferase (OCT) 2.10; aldolase 0.75.
Fig. 3.—Initial appearance of the eruption.

Fig. 4.—Later appearance of the eruption.

On June 30th, the patient’s temperature fell to 37 C., his appetite returned and he commenced to gain weight. On July 4th, the patient left the aseptic room and the hospital; treatment with INH, streptomycin and Δ-1-cortisone was continued.

Establishing the Existence of a True Hemopoietic Graft and Chimerism

Several different methods were used to establish the manner and extent to which hemato-poietic restoration was due to the grafting of transfused cells.

(a) The study of chromosomes in the circulating leukocytes showed no lesions such as
those we described in a patient who received the same dose of radiation\textsuperscript{35} and who was later given infusions of isogenic bone marrow. In that patient there was only a temporary acceptance of the graft, followed by autologous marrow recovery. The absence of the chromosomal lesions seen following x-irradiation suggested that the blood cell population consisted entirely of nonirradiated foreign cells.

(b) The phenotype of the patient’s erythrocytes became the phenotype of the donor P; nearly 100 per cent of red cells were agglutinated by anti-s serum and by anti-Le (a +) serum, whereas no red cell agglutinated with anti-c or anti-S serum. Studies of the leukocyte sex chromatin demonstrated a few female cells: 0 to 4 per cent of polymorphonuclears showed drumsticks and 7 to 41 per cent of mononuclears, heterochromatic lobules; 3 per cent of the mitoses had a female karyotype. The graft, therefore, consisted mostly of P cells with a small proportion of female donor cells.

(c) The Inv groups shown by the patient after treatment were: Inv (1-a-b +) (Dr. Ropartz\textsuperscript{37}); thus, the host began to produce beta 2A, beta 2M and gamma globulins of the type of one or more of the donors.

Specific Tolerance due to Chimerism

On June 6th, the patient received skin grafts from the various marrow donors and from himself. Unfortunately, the graft from donor F (sister) was torn off with a dressing. The development of the other grafts were as follows: the graft from D was cast off on the 15th day, that from M on the 24th day, that from G on the 28th day and that from H not before the 60th day. The autograft and the graft from P were perfectly intact after seven months (fig. 9) demonstrating the existence of specific tolerance to P.

Reactivation of the Secondary Syndrome

In order to enhance the reaction of the graft against the host (with the aim of increasing its eventual anti-leukemic effect) the patient received on October 1st, 8th, 15th and 22nd, 1963, 29 x 10\textsuperscript{6}, 49 x 10\textsuperscript{6}, 40 x 10\textsuperscript{6} and 45 x 10\textsuperscript{6} isolated leukocytes from the donor.
P whose bone marrow had been successfully grafted. From the time of the first injection, symptoms and signs again appeared suggesting a recurrence of the secondary syndrome: pruritus, erythrodermia, and hepatomegaly with serum enzyme alterations (on November 18th the serum aldolase was 142, SGOT 146, SGPT 410, OCT 2.85). Liver biopsy showed hepatic cells moderately rich in glycogen and a few distended bile capillaries with bile thrombi. There was a pronounced increase in Kupfer cells, with intralobular foci of histiocytic proliferation. At the margin of one of these foci, a single possibly necrotic liver cell was seen. Slight periportal infiltration with lymphoid cells, mostly histiocytes, was found. All of the above signs and symptoms regressed after treatment with Δ1-hydrocortisone.
Fig. 8.—Cytologic aspects of the smear of the lymph node obtained on June 1st: note the paucity of lymphocytes; numerous histiocytes, often hyperbasophilic and the presence of eosinophil granulocytes. May Grünwald-Giemsa.

DISCUSSION

Our previous experience with these technics in man includes 14 attempts at allogenic bone marrow transplantation after total-body irradiation at lethal dosage (six other attempts were made after sublethal irradiation). Varying results were obtained:

(a) Some transfusions of bone marrow to leukemic patients were not followed by grafting and the subjects died of aplasia.\(^30\)\(^31\) We studied the cause of these failures and found indications of possible isoimmunization due to prior transfusions. Experiments in mice, done jointly with Da Costa,\(^8\) confirmed that blood transfusions given before irradiation, considerably reduce the chance of graft acceptance.

(b) Marrow transfusions given to subjects inadvertently irradiated (at Vinca, Yugoslavia) with neutrons and gamma rays were followed by a temporary take lasting 3 weeks with no secondary syndrome.\(^32\) The reasons for this may have been that the transfusions were given rather late relative to the irradiation and that the radiation each subject received (between 75 and 100 per cent of a lethal dose\(^26\)) was not homogenous.

(c) Some bone marrow transfusions in leukemic subjects were followed by a graft which was more persistent (3 months) than that in the accidentally irradiated cases and was complicated only by a minor secondary syndrome.\(^30\) Although the anti-leukemic effect was notable (a 1-year remission was obtained in one patient and an 8-month remission in another), the impression was that the maximal graft versus host reaction had not transpired.

(d) Other attempts in leukemic subjects led to more complete grafts, complicated by early and very severe secondary syndromes which could not be
controlled; the patients died despite excellent myeloid restoration. Histologic, hematologic and biochemical studies showed the similarity of this human secondary syndrome with that in animal species, particularly monkeys.

The optimum therapeutic result, we felt, would be obtained by the induction of a persistent graft, followed by a marked but controllable secondary syndrome. In the light of our previous failures in achieving prolonged bone marrow homografts, we have considered several aspects of the present case and their possible contribution to its apparent success:

(1) It is noteworthy that the patient was able to tolerate a prolonged period of virtually total myelo-lymphoid aplasia without serious effect. This could not have been accomplished without meticulous aseptic hospital care and the prevention of serious hemorrhagic complications with repeated platelet infusions and the administration of epsilon-amino-caproic acid.

(2) The irradiation dose of 800 r. preceded by the administration of methyl-nitro-imidazolyl-mercaptopurine for 4 days was apparently sufficient to condition the patient; however, this dose of radiation alone, without concurrent administration of antimitotic products, had previously permitted us to obtain allogenic bone marrow grafts in other leukemic subjects. It is impossible to know, therefore, whether the addition of methyl-nitro-imidazolyl-mercaptopurine (Imuran®) was, in fact, useful. Its administration before irradiation seemed logical, because antipurines are known to inhibit immune responses when given before the antigen, more than when given afterwards. Moreover, Cole et al. found it effective and well tolerated in the dog.

(3) As already mentioned, we attach considerable importance to the fact
that our subject had never previously received a blood transfusion before ir-
radiation.

(4) The use of multiple donors in this case was of twofold importance: (a) it made it possible to administer a total number of cells (5.8 x 10^10 in this case), considerably larger than that which can be obtained from a single donor (10^10 at best, in our experience); and (b) it permitted a spontaneous selection by the recipient of the best of several most closely related donors, with a consequent milder secondary syndrome, as in the mouse.22,23 It should be pointed out that P, the accepted donor, showed a histocompatibility test which characterized him as nearest in relation to the recipient.24,33 The mar-
row from the other donor indicated by the test (H), was probably also pro-
tractedly tolerated since his skin graft was not rejected until the 60th day. The test placed F in the third position. A study of leukocyte antigens also in-
dicated that P was the best donor and F and H good donors. In this regard, the erythrocytic and serum phenotypes appeared to be of no importance.

This method would seem to be especially suitable for the creation of a tolerance associated with chimerism in man. If, as the general failure of numerous attempts made after conditioning by subtotal irradiation or by means of antimitotics indicates10,13,15,16,24 that the hope of a lasting success of trans-
plantation of allogenic kidneys rests upon the tolerance associated with the hemopoietic chimerism,14 then the multiple donor method will make possible not only the success of the first stage, namely, the establishment of the chimer-
ism, but also the choice of the best donor by the study of the behavior of skin grafts from various bone marrow donors.

(5) This patient showed all the cardinal symptoms of the secondary syn-
drome, which was severe, early and prolonged (10 weeks), yet controllable. It ranked in severity between the early, fatal syndromes31 and the late minor ones30 previously described in man, as well as in various animals, e.g., mouse,7,14,19,41 rabbit,36 dog,11 and monkey.4 The outstanding clinical symp-
tom was fever, of variable intensity, which ensued considerably before the miliary disease occurred; also present was marked progressive weight loss, anorexia, nausea with vomiting, capricious diarrhea with no digestion of foods, hepatomegaly, transient polyadenopathy and particularly desquamating erythrodermia. The liver function abnormalities and the marked eosinophilia were not, in themselves, characteristic of the secondary syndrome. The two biopsies carried out (lymph node and skin) appeared to contain typical secondary syndrome lesions, viz.: (a) in the lymph node, the lymphocytic aplasia and hyperbasophilic histiocyte proliferation which are described as characteristic of the secondary syndrome by Binet and Mathé;5 (b) in the skin, the dyskeratosis, vacuolization of basal cells and dermal infiltration by lymphoid cells.

(6) The reactivation of the signs of the secondary syndrome by injections of leukocytes from the donor whose bone marrow was tolerated by the host favors the idea that the secondary syndrome is, in man as in the animal, due to the graft versus host reactions.

(7) Prior to the described therapeutic attempt, the patient was in leukemic
relapse, refractory to most of the treatments available (steroids, antipurines, leurocristine). At the present time, an apparently complete remission has existed for 12 months; blood and bone marrow are completely normal; no lymphoblasts can be seen. More time is required to define the extent to which a severe, controlled secondary syndrome exerts a therapeutic effect in spontaneous human leukemia. It is, of course, impossible to dissociate the importance of total-body irradiation from that of the reaction of the graft to the leukemic cells. The complete remission obtained in this patient constitutes a noteworthy result.

SUMMARY

A patient suffering from acute lymphoblastic leukemia in the third progressive phase, refractory to the majority of available treatments, was given total-body irradiation of 800 r. dosage, after administration of methyl-nitro-imidazolyl-mercaptopurine (300 mg. daily for 4 days). One week after irradiation, he received an infusion of a mixture of equal parts of bone marrow from six donors (mother, sister, father and three brothers), a total of 5.8 x 10^{10} nucleated cells.

Myeloid restoration began 2 weeks after the infusion and continued to progress rapidly. The reality of the graft was demonstrated by study of the erythrocytic antigens. Nearly all the erythrocytes found in the blood after 6 months belonged to the phenotype of one of the male donors.

Studies of chromosomes and leukocyte sex chromatin suggested that a
small proportion of leukocytes were being produced by the cells of one of the female donors. The patient was found to produce beta 2A, beta 2M and gamma globulins of the Inv type of the donors, suggesting that lymphoid cells may also have been successfully grafted.

A skin graft from the male donor whose marrow graft had apparently been accepted was still perfectly intact 7 months after grafting, whereas the grafts from other donors were rejected, indicating a specific tolerance to tissues from this donor. Previous studies of compatibility between the recipient and the various donors by a histocompatibility test described by us, or by means of similarities in leukocyte antigens, indicated that the accepted donor had been antigenically more nearly compatible than the others.

What appeared to be a secondary syndrome became manifest 1 week after marrow transfusion; it consisted of weight loss, digestive disorders (anorexia, nausea, vomiting, diarrhea), hepatic disturbances (hepatomegaly, increased serum concentrations of various enzymes), transient polyadenopathy (histologically composed of histiocytic proliferation and lymphocytic aplasia), desquamative erythrodermia, eosinophilia exceeding 50 per cent, and super-infection (including a probable miliary tuberculosis). The syndrome was controlled by careful symptomatic treatment; its intensity gradually abated.
 Reactivation of the secondary syndrome was accomplished by reinjection of leukocytes from the donor whose graft had been tolerated. This was controlled by Δ-1-cortisone and symptomatic treatment.

The patient was still alive and in apparently complete hematologic remission from the leukemia 12 months after the treatment described above. Particular reasons for this success possibly include the absence of previous blood transfusions and the use of multiple marrow donors.

ADDENDUM

Since this paper was submitted for publication in November, 1963, the patient remained well until January, 1964, when he suffered from a widespread necrotic herpes zoster, complicated by meningo-encephalitis, which was associated with a right hemiplegia. This lesion slowly improved over a period of 5 months, but after this he was troubled by marked psychiatric disturbances with attacks of hypersomnia, weeping, sexual obsessions, and recurrent flushing of the face. They were considered to be typical of viral encephalitis (Dr. Borenstein). The patient died on December 17, 1964 in a coma after a convulsive episode.

P. M. (Dr. M. J. de Vries) 20 months after the irradiation. There was no evidence of any leukemic infiltration of the organs. Bone marrow slightly hypocellular with some infiltration with lymphoid cells. Lymphoid follicles were present in the spleen and intestinal lymphoid tissue.

Multiple foci of infiltration with lymphoid cells, plasma cells and histiocytes were present in the prostate, periportal spaces of the liver and perivascularly in the spinal cord.

The characteristics of secondary disease were not seen in the intestines; however, the multiple foci of infiltration would suggest a chronic graft versus host activity.

The cause of death appeared to be due to acute pulmonary congestion, complicating an encephalitis.

ACKNOWLEDGMENTS

We gratefully acknowledge the help received from Drs. P. Borenstein, Y. Cachin, J. Dausset, P. Denoix, G. Hitchings, C. Ropartz, C. Salmon, Mrs. C. Brézin and F. Lapeyraque.

SUMMARIO IN INTERLINGUA

Un patiente qui suflreva de acute leucemia lymphoblastic in le tertie phase progressive (refractori contra le majoritate del dispobilis formas de tractamento) esseva subjicite a irradiation del corpore total in un dosage de 800 r, precedite per le administration de quatro consecutive doses diurne de 300 mg de methyl-nitro-imidazolyl-mercaptopurina. Un septimana post le irradiation, le patiente recipeva un infusion de un mixtura de partes equal de medulla ossee ab sex donatores (matre, soror, patre, e tres fratres), amontante a un total de $5.8 \times 10^{10}$ cellulas nucleate.

Le restauration myeloide comenciava duo septimanas post le infusion e continuava progredier rapidermente. Le realitate del graffo esseva demonstrate per le studio del antigenos erythrocytic. Quasi omne le erythrocytos trovate in le sanguine post un intervallo de sex menses pertineva al phenotypo de un del donatores mascule.

Studios de chromosomas e del leucocytic chromatina sexual pareva indicar que un micro proportion de leucocytes esseva produce per le cellulas de un del donatores feminin. Esseva trovate que le patiente produceva globulinas
ALLOGENIC BONE MARROW TRANSPLANTATION

beta 2A, beta 2M, e gamma del typo Inv del donatores. Isto suggere que cellulas lymphoide esseva forsan etiam graffate a bon successo.

Un graffo cuntanee ab un del donatores mascule—ille ab qui le graffo de medulla habeva apparentemente essite acceptate—esseva ancora perfectemente intacte septe menses post le transplantation, durante que simile graffos ab le altere donatores esseva rejicite. Isto indica un tolerantia specific protissu ab le mentionate donator particular. Previe studios de compatibilitate inter le recipiente e le varie donatores per medio de un test de histocompatabilitate (describebe per nos) o per medio de constatationes de similitudes in antigenos leucocytic indicava que le duo acceptate donatores habeva essite plus approximativamente compatibile ab le puncto de vista de antigenicitate que le alters.

Un syndrome, apparentemente de character secundari, se manifestava un septimana post le transfusion de medulla. Illo consisteva de perdita de peso, disordes digestive (anorexia, nausea, vomito, diarrhea), disturbationes hepatic (hepatomegalia, augmento del concentrationes seral de varie enzimas), transiente polyadenopathia (histologicamente componite de proliferation histioctic e aplasia lymphocytic), erythrodermia desquamative, eosinophilia in excesso de 50 pro cento, e superinfection (incluse probabilemente tuberculosis miliari). Le syndrome esseva combattite per un meticulose tractamento symptomatic. Su intensitate recedeva gradualmente.

Reactivation del syndrome secundari esseva complite per le reinjection de leucocytos ab le donator ab qui le graffo habeva essite tolerate. Isto esseva combattite per delta-1-cortisona e tractamento symptomatic.

Le patiente esseva vive e apparentemente in complete remission hematologic de su leucemia 12 menses post le supra-describebe tractamento. Le rationes particular de iste successo include possiblemente le absentia de previe transfusiones de sanguine e le utilisation de un multiplicitate de donatores de medulla.

REFERENCES
8. Da Costa, H., Amiel, J. L., and Mathé,
leucémie L 1210 par l’irradiation X
suivie de transfusion de cellules hémato- 
apoïétiques normales (isologues ou
homologues, myéloïdes ou lymphoïdes,
adultes ou embryonnaires). Rev.
30. — —, Schwarzenberg, L., Larrieu, M. 
J., Lalanne, C., Dutreix, A., Denoix, 
P., Surmont, J., Schwarzmann, V.,
and Ceoara, B.: Essai de traitement
de sujets atteints de leucémie aigue
en rémission par l’irradiation totale
suivie de transfusions de moelle os-
seuse homologue. Rev. Française et
31. — —, de Vries, M. J., Schwarzenberg,
L., Larrieu, M. J., Lalanne, C., Du-
treix, A., Amiel, J. L., and Surmont,
J.: Nouveaux essais de greffe de
moelle osseuse homologue après irra-
diation totale chez des enfants atteints
de leucémie aigue en rémission. Le
problème du syndrome secondaire chez
32. —, Jammet, H., Pendic, B., Schwarzen-
berg, L., Duplan, J. F., Maupin, B.,
Latarjet, R., Larrieu, M. I., Kalic, D.,
and Djuric, Z.: Transfusions et greffes
de moelle osseuse homologue chez
des humains irradiés à haute
dose et traités par transfusion de
33. Matsukura, M., Mery, A. M., Amiel, J.
L., and Mathé, G.: Investigation on a
test of histocompatibility for allogenic
grafts. II. A study on rabbits. Trans-
34. Murray, J. E., Merrill, J. P., Dammain,
G. J., Dealy, J. B., Alexandre, G. W.,
and Harrison, J. H.: Kidney trans-
plantation in notified recipients. Ann.
35. Papiernik, M., Amiel, J. L., and Mathé,
G.: Anomalies chromosomiques après
irradiation totale chez l’Homme. C.
36. Porter, K. A.: Graft-versus-host reaction
in the rabbit. Brit. J. Cancer 14:66,
1960.
37. Ropartz, C.: Données actuelles sur les
groupes héréditaires de γ globulines. 
38. Salmon, C.: Technique d’appréciation
quantitative de 2 populations d’héma-
ties chez quatre sujets irradiés à haute
dose et traités par transfusion de
moelle osseuse. Rev. Française et
39. Seman, G.: Technique personnelle de
coloration des lobules hétérochro-
matiques des leucocytes, spécialement
des mononucléés. Valeur de cette
technique pour la détermination du
40. de Vries, M. J., Crouch, B. L. G., van
Putten, L. M., and van Bekkum, D.
W.: Pathologic changes in irradiated
monkeys treated with bone marrow.
41. de Vries, M. J., and Vos, O.: Delayed
mortality of radiation chimeras: a
pathological and hematological study.
M. Schneider, Assistant du Service d'Hématologie de l'Institut Gustave-Roussy, Villejuif, France.

M. J. de Vries, Pathologist, Radiobiological Institute, Rijswijk, Holland.

M. Tubiana, Chef de Service à l'Institut Gustave-Roussy, Villejuif, France.

C. Lalanne, Chef de Service à l'Institut Gustave-Roussy, Villejuif, France.

J. L. Binet, Chef de Clinique de la Faculté de Médecine de Paris, France.

M. Papiernik, Assistant de l'Institut de Cancérologie et Immunogénétique, Villejuif, France.

G. Seman, Assistant de l'Institut de Cancérologie et Immunogénétique, Villejuif, France.

M. Matsukura, Assistant étranger de l'Institut de Cancérologie et Immunogénétique, Villejuif, France.

A. M. Mery, Assistant de l'Institut de Cancérologie et Immunogénétique, Villejuif, France.

V. Schwarzmann, Chef de Laboratoire, Hopital Saint-Antoine, Paris, France.

A. Flaisler, Assistant à l'Institut Gustave-Roussy, Villejuif, France.
Successful Allogenic Bone Marrow Transplantation in Man: Chimerism, Induced Specific Tolerance and Possible Anti-Leukemic Effects


Updated information and services can be found at: http://www.bloodjournal.org/content/25/2/179.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at: http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at: http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at: http://www.bloodjournal.org/site/subscriptions/index.xhtml