Adoptive Immunotherapy of Acute Leukemia: Experimental and Clinical Results[1]

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SUMMARY

The theoretical principle of treatment of acute leukemia by adoptive immunotherapy is to permit allogenic, immunologically competent cells to act against the host's leukemic cells and the basic leukemogenic factors present in the host. Such an effect may be obtained by a graft of allogenic hematopoietic cells following total-body irradiation. If the patient is able to survive the secondary syndrome that is induced by the homograft, an eradication of the leukemia may well be obtained. It has been demonstrated that this mode of therapy is effective in the treatment of spontaneous, transplanted, and viral leukemias in mice. Clinical trials of adoptive immunotherapy of acute leukemia in man are described. The results show that it is possible to obtain a sustained graft of hematopoietic cells and that such a graft has a distinct value as an anti-leukemic agent.

The impossibility of controlling acute leukemia is explained by a number of reasons; including the facts that (*a*) the available means cannot eradicate all leukemic cells; (*b*) it is possible that human acute leukemia, like some leukemias of mice, is caused by a virus whose persistence should be held responsible for the recurrence of the disease after each remission; (*c*) the hemopoietic tissues are spread through the entire organism, which prohibits their eradication by surgical excision or high-dosage local radiotherapy; these tissues may, during remissions, carry either leukemic cells or apparently normal elements capable of producing new leukemia by the carcinogenic factor (particularly a virus), or both.

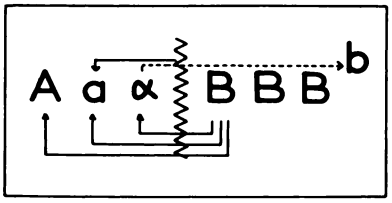


CHART 1.—Theoretical design of the basis of the adoptive immunotherapy of acute leukemia. The symbols are explained in the text.

In the following therapeutic experiment, an attempt is made to overcome these three stumbling blocks. Let subject *A* (Chart 1) carry leukemic cells *a*, the leukemia possibly being caused by virus α. The subject is submitted to total irradiation at the maximum dosage tolerated; this dose considerably reduces the number of leukemic cells but cannot eradicate the neoplasm (about 4 times this dose would be required [2]). This dosage, however, conditions the subject as recipient of an allogenic bone marrow graft from subject *B* (*cf.* [9]). The myeloid cells of this bone marrow (erythroblasts, myeloblasts, megakaryoblasts) repopulate the bone marrow and blood which were depopulated by irradiation. The immunologically competent cells react to various antigenic elements which they encounter, in particular the leukemic cells *a* (1, 4, 10, 12, 14, 15) and possibly virus α (11; G. Mathé, unpublished data), thus effecting an anti-leukemic immunotherapy. Unfortunately, they simultaneously react to the recipient's normal antigens and thus produce the "secondary syndrome" (*cf.* [9]). If the subject survives this syndrome, and if the additional effect of irradiation against the leukemic cells *a* (the more intensive the radiation, the more cells are reduced [20]) and that of the immunologic reaction have eradicated all the cells, then it is not unreasonable to hope that the existing leukemia can be controlled; nor is it un-

TABLE 1

ADOPTIVE IMMUNOTHERAPY OF A TRANSPLANTED LEUKEMIA (L1210) IN MICE; RECIPIENTS $D2B6F_1$

Treatment	No. of mice	Reaction	Mean survival (days)	No. of leukemia-free survivors 200 days after graft
Controls	78	None	11	70
850 r total irradiation and F_1 bone marrow graft	70	−	14.4	0
850 r total irradiation and C57BL6 bone marrow graft	54	+	22.6	1

[1] This study was carried out with the aid of CA 05703-04 RAD, NIH, Bethesda, Maryland.

reasonable to hope that the virus α could be eradicated by the immune reaction or that its concentration could be greatly reduced; or, if it is a virus specific of "strains" such as some leukemogenic viruses of mice (Gross' virus [6], Charlotte Friend's [5]), if the grafted *B* marrow produces 100% hemopoietic, medullary, and lymphoid cells, and if this graft is permanent, it will not be able to produce a leukemia *b* on the basis of *B* cells.

The object of this paper is to submit this hypothesis to experimental study, with mouse leukemia, and to clinical trials.

TABLE 2

ADOPTIVE IMMUNOTHERAPY OF A TRANSPLANTED LEUKEMIA (L1210), WITH CONTROL OF THE SECONDARY SYNDROME BY MEANS OF ANTIMITOTICS, IN MICE

Each mouse received 850 r and 10^7 allogenic marrow cells, plus the drug indicated below.

Drug	No. dying from leukemia	No. dying from secondary syndrome	No. surviving without leukemia 200 days after graft	Total
None	39	9	1	49
Amethopterin[a]	43	3	4	50
Cyclophosphamide[b]	46	6	5	57

[a] Two mg/kg every other day from the 15th to the 33rd day.

[b] One hundred eighty mg/kg every other day from the 15th to the 33rd day.

EXPERIMENTAL PROCEDURE

Transplanted leukemia.—The experiments (12, 15) were made in C57BL6 × DBA2 F_1 mice (hereafter called B6D2F_1) given a graft of 10^4 cells of L1210 leukemia. Four hours after the transplantation the mice were submitted to total irradiation of 850 r (200 kv, 12 ma, 50 cm, 0.5 Al, 0.5 Cu). Four hours after irradiation, 10^7 medullary cells were transplanted by the venous route; these cells were obtained from C57BL6 donors. The results, which were compared with those obtained by transplantation of bone marrow from B6D2F_1 donors, can be described as follows.

As Table 1 shows, the mean survival was longer when the irradiation was associated with an immune reaction of C57BL6 cells against the DBA2 leukemic cells. One

TABLE 5

ADOPTIVE IMMUNOTHERAPY OF VIRAL LEUKEMIA (FRIEND) IN D2CBAF_1 MICE

Treatment	Total No. of mice used	No. of deaths from leukemia
0.2 ml blood from isogenic mice given inoculations of Friend virus and treated with total irradiation and isogenic marrow	31	31
0.2 ml blood from isogenic mice given inoculations of Friend virus and treated with total irradiation and allogenic marrow C57BL6	21	14

TABLE 3

ADOPTIVE IMMUNOTHERAPY OF SPONTANEOUS AKR LEUKEMIA AT THE AGE OF 6 MONTHS

Group	Survival after irradiation		Total survival		Frequency (%)	
	Mean	Maximum	Mean	Maximum	Secondary syndromes	Leukemia
Control (nonirradiated)			8 mo 18 days	14 mo		89
Irradiated	11 days	14 days	6 mo 11 days	6 mo 14 days		0
Irradiated and restored with hematopoietic cells						
C3H adult	1 mo 19 days	7 mo 20 days	7 mo 19 days	13 mo 20 days	57	29
C3H fetal	1 mo 17 days	13 mo 4 days	7 mo 17 days	13 mo 4 days	53	19
C57BL6 adult	1 mo 7 days	6 mo 6 days	7 mo 7 days	13 mo 6 days	63	17
C57BL6 fetal	1 mo 14 days	4 mo 28 days	7 mo 14 days	10 mo 28 days	67	14

TABLE 4

ADOPTIVE IMMUNOTHERAPY OF VIRAL LEUKEMIA (FRIEND) IN DBA2 MICE

Group A—mice inoculated but not treated; Group B—mice inoculated, irradiated, and restored with isogenic marrow from nonvaccinated donors; Group C—mice inoculated, irradiated, and restored with isogenic marrow from vaccinated donors.

Group	No. of animals inoculated	No. of mice with leukemias	No. of mice surviving on 250th day	Median survival of leukemic mice (days)	Mean survival of leukemic mice and confidence interval of mean if $P = 0.05$ (days)
A	18	18	0	83	86.1 (75.6- 96.6)
B	12	7	1	52	70.0 (37.7-103.3)
C	8	8	0	163	163.7 (147.2-180.2)

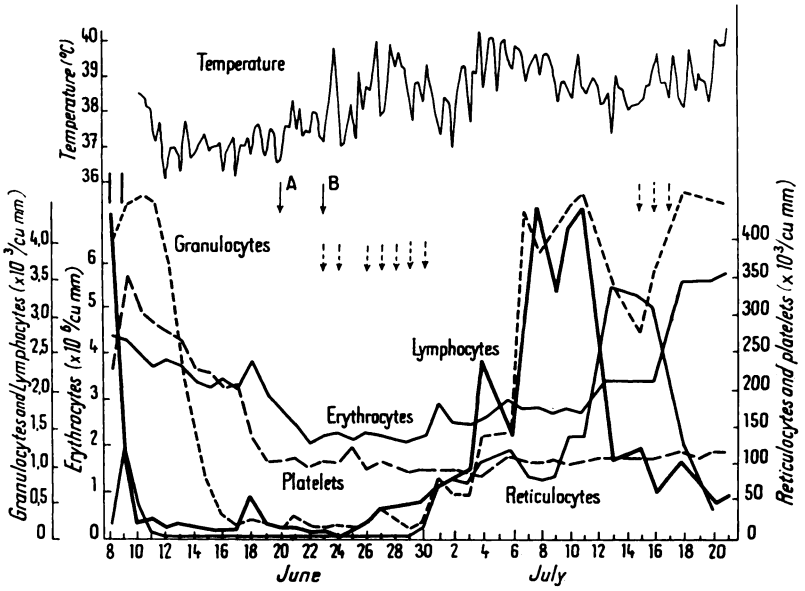


CHART 2.—Changes in body temperature and blood count during the phase of aplasia and the acute secondary syndrome in a patient irradiated with 800 rad and treated by allogenic bone marrow transfusions.

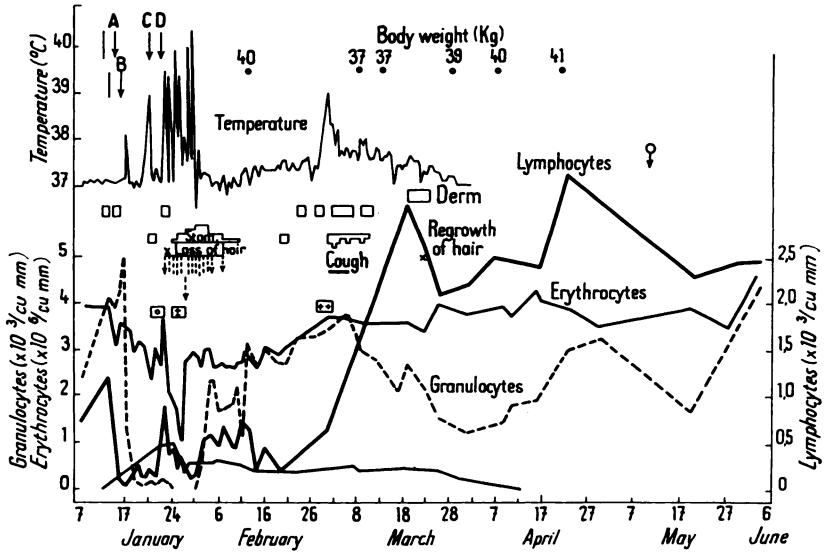


CHART 3.—Clinical events and hematologic changes during the phase of irradiation aplasia and chronic secondary syndrome in a patient irradiated with 800 rad and treated by allogenic bone marrow transfusions.

mouse in the experiment indicated in Table 1 was cured. At postmortem, 35% of the mice which died after the 20th day showed no sign of leukemia but had histologic lesions of the secondary syndrome. This experiment was repeated several times, always with similar results.

In another experiment (12), in which the secondary syndrome was treated with amethopterin or cyclophosphamide in doses ineffective against leukemia (2 mg/kg and 180 mg/kg, respectively, every other day, from the 15th to 33rd day after irradiation), the number of "cured" mice was larger; statistical studies proved that the action of these drugs is confined to the secondary syndrome (Table 2).

Other authors have also described this immunotherapeutic action against leukemia (1, 4) and other transplanted tumors (21).

Spontaneous leukemia.—Similar experiments, involving total irradiation at lethal dosage followed by transplantation of allogenic bone marrow, were carried out with spontaneous AKR leukemia.

In one experiment (14), the animals were treated at an advanced stage, and results were disappointing because the majority of animals died after treatment.

In a second experiment (10), the mice were systematically treated at the age of 6 months (only 24% of controls at that age showed histologically verifiable leukemia). The animals received 750 r irradiation, followed by transplantation of hemopoietic cells from donors C3H or C57BL6 (adults or fetuses, the cells in the latter case being obtained at the age of 17 days). The results (Table 3) can be described as follows. The mean survival was not prolonged but, in fact, was shorter than in controls; at post-

TABLE 6

ERYTHROCYTE PHENOTYPES OF THE HOST AND OF THE DONORS

Host B. B. before injection of allogenic bone marrow	O, N/S, P_1, CcDee, K−, Kp(a−), Le(a−b+), Fy(a+), Jk(a+)
Donors	
H. B.	O, N/S, P_1, CcDee, K−, Kp(a−), Le(a−b+), Fy(a+), Jk(a+)
D. B.	O, N/S, P_1, CcDee, K−, Kp(a−), Le(a−b+), Fy(a+), Jk(a−)
M. B.	O, N/S, P_1, CcDee, K−, Kp(a−), Le(a+b−), Fy(a+), Jk(a−)
P. B.	O, N/ss, P_1, CCDee, K−, Kp(a−), Le(a+b−), Fy(a+), Jk(a+)
G. B.	O, N/S, P_1, CCDee, K−, Kp(a−), Le(a−b+), Fy(a−), Jk(a+)
F. B.	O, N/S, P_2, CCDee, K−, Kp(a−), Le(a−b+), Fy(a+), Jk(a+)
Host B. B. 2 months after injection of allogenic bone marrow	O, N/*ss*, P_1, C*C*Dee, K−, Kp(a−), *Le*(*a*+*b*+), Fy(a+), Jk(a+)

TABLE 7

SERUM PHENOTYPES OF THE HOST AND OF THE DONORS

Host B. B. before injection of allogenic bone marrow	Gm(a−b+x−e+), Inv (1+a+b+)
Donors	
H. B.	Gm(a−b+x−e+), Inv (1−a−b+)
D. B.	Gm(a−b+x−e+), Inv (1−a−b+)
M. B.	Gm(a−b+x−e+), Inv (1−a−b+)
P. B.	Gm(a−b+x−e+), Inv (1−a−b+)
F. B.	Gm(a−b+x−e+), Inv (1−a−b+)
Host B. B. 2 months after injection of allogenic bone marrow	Gm(a−b+x−e+), Inv (*1*−*a*−*b*+)

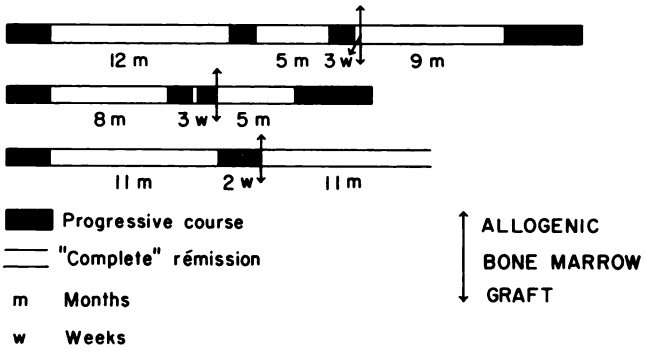


CHART 4.—Summary of the results obtained in 3 patients with acute lymphoblastic leukemia by treatment with allogenic bone marrow grafts.

mortem, however, the majority of mice treated showed no histologic sign of leukemia; they had various lesions of the secondary syndrome, which must be held responsible for the deaths.

Viral leukemia.—Experiments were carried out with the Friend leukemia. In the first experiment (11), DBA2 mice were treated, 3 days after inoculation of a 100% leukemogenic dose of virus, by total irradiation with 700 r, followed 4 hr later by transplantation of bone marrow from isogenic donors, either normal or previously vaccinated with formaldehyde-treated virus. Table 4 shows that the animals treated with bone marrow from donors thus immunized against the virus showed a survival rate exceeding that in animals given bone marrow from untreated donors; this indicates the anti-leukemic effect of the anti-viral immunologic reaction.

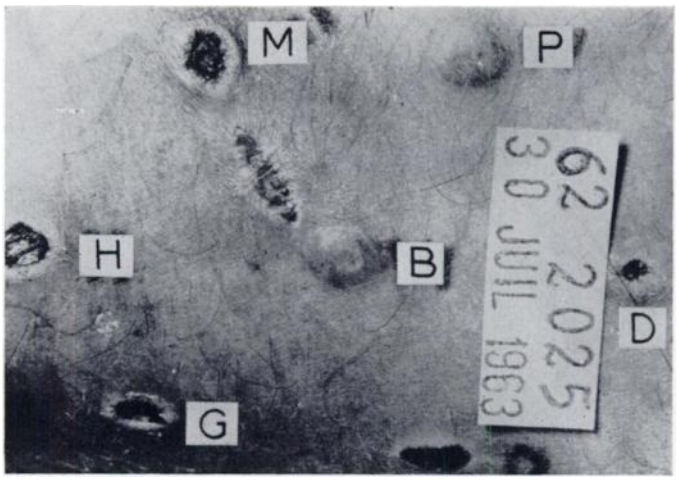
DBA2 × CBA F_1 mice (hereafter called D2CBAF_1) were treated, 4 hr after virus inoculation, by total irradiation with 950 r followed by transplantation of 10^7 isogenic or allogenic medullary cells C57BL6 4 days later; 0.3 ml of blood was obtained and injected i.v. into D2CBAF_1 mice. In the group given inoculations of the blood from mice treated with isogenic bone marrow, 100% of the animals died of leukemia. In the group given inoculations of blood from mice treated with allogenic bone marrow, 33% of the animals showed no leukemia (Table 5), which indicates that active virus may be eradicated in the blood of allogenic radiochimeras (or its concentration lower than in isogenic).

CLINICAL TRIALS

Our experience with clinical trials of adoptive immunotherapy by transfusion of allogenic hemopoietic cells concerns 21 patients with acute lymphoblastic leukemia. We exclude from this paper the 3 latest trials, the study of which is still in progress, and 8 trials (7) carried out under unfavorable conditions, either because the patients had reached the terminal stage of illness or because the dose of irradiation used was only sublethal and did not correspond with the criteria based on experimentation. Only after therapeutic experiments with bone marrow grafts in 5 subjects accidentally irradiated with a dose between LD_{75} and LD_{100} (19) did we apply 100% lethal doses.

Ten cases in which the trials corresponded with the conditions of the animal experiments are available for analysis. Irradiation with γ-rays from ^{60}Co was given up to 800 r in two 400-r sessions at an interval of 12–24 hr. The

FIG. 1.—Skin grafts from various donors 4 months after bone marrow transplantation in a patient (*B*) irradiated with 800 r, treated with the bone marrow of these donors, and restored by a marrow graft from 1 of them (*P*). Tolerance of the autogenous graft and of a skin graft from *P* was the same after 12 months.



interval until transplantation of allogenic marrow was 3–14 days, and the number of cells varied from 1.09×10^9 to 5.8×10^{10} (the number of donors was 1–6).

RESULTS

Three patients died of myeloid aplasia 20–35 days after irradiation (16, 17), the bone marrow graft being shed. One explanation for these failures includes the possible (cross) immunization by blood transfusion given before irradiation; this hypothesis is borne out by the results of experiments in mice (3).

Four patients died after 30 days in spite of the taking of the graft; death was ascribed to the acute and very severe secondary syndrome, which showed clinical, hematologic (Chart 2), biochemical, and histologic features entirely identical with those observed in other animal species (8, 16, 18). None of these patients showed post-mortem signs of leukemia.

Three patients survived the secondary syndrome, which was late and moderate in two of them (Chart 3) (17), probably because of the partial, transient nature of the graft; it was early and severe in the third, who, 11 months after transplantation, showed 100% red cells (Table 6) which had the antigenic characteristics of the donor cells, as well as immunoglobulins which showed the genetic characteristics of the donors (Table 7), tolerating a skin graft from the donor whose graft was functioning (Fig. 1) (13).

Apparently complete remission was obtained in the 1st 2 patients; the 1st, with a 26-month history of leukemia and 3 remissions lasting 12 months, 5 months, and 3 weeks, respectively, survived 12 months, including 9 months of complete remission. The 2nd, with a 13-month history of leukemia with 1 remission of 8 months and 1 of 3 weeks, had a relapse at the time of the trial and survived 9 months, including 5 months of complete remission. The third patient, with a 20-month history of leukemia and 1 remission of 11 months and 1 of 2 weeks, was in complete remission 11 months after irradiation (Chart 4).

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We are indebted to Drs. M. Tubiana and C. Lalanne, who carried out the total irradiation of our patients.

ADDENDUM

As this article was written in March 1964, we think it useful to add that the case of the patient for whom an 11-month survival was indicated has been published in detail in *Blood* (*25:* 179, 1965). It may be seen in this publication that the patient died of herpes zoster encephalitis at the 20th month. The hematologic and necropsy findings at that time revealed no leukemic infiltration.

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