Haematopoietic Chimera in Man After Allogenic (Homologous) Bone-marrow Transplantation

(Control of the Secondary Syndrome. Specific Tolerance Due to the Chimerism)*

In 1958 we were able to demonstrate the possibility of transient grafting of allogenic bone-marrow in the human subject accidentally exposed to whole-body irradiation,[28] and between 1959 and 1963 we confirmed this possibility in patients with leukaemia who were irradiated intentionally.[26 27] In the latter series of experiments there have been several cases of failure, which after a subsequent experimental investigation[8] we are inclined to attribute to a cross-immunization resulting from previous blood transfusions. In those cases where grafts were found to take, some were relatively well tolerated—that is, they were complicated by a secondary syndrome which was of moderate intensity—but of a rather brief duration (approximately three months),[26] whereas others were complicated by a secondary syndrome that ran a rapidly fatal course.[27]

During a recent experiment in mice we found that the use of a mixture of bone-marrow from several donors offers certain advantages: the frequency of the myeloid restoration and the incidence of the secondary syndrome in such cases are approximately the same as can be observed, for the same number of transfused cells, with the bone-marrow of the donor who has shown the best histocompatibility; the cause of this is that the host organism spontaneously selects the best donor, as could be demonstrated in a study of the specific tolerance due to chimerism.[19 20] These observations have guided us in the test case reported below.

TEST CASE†

The patient (B. B.) was a man aged 26 who had never had any blood transfusions. Since August, 1961, he had suffered from acute lymphoblastic leukaemia, which was in a relapse phase; the condition was resistant to adrenocortical steroids, to mercaptopurine, and to leurocristine. On April 17 and 18, 1963, he was subjected to whole-body irradiation with cobalt-60 γ-rays, in a dose of 800 rad (two sessions of 400 rad), preceded by four days' administration of methyl-nitro-imidazolyl-mercaptopurine (Burroughs Wellcome) in doses of 300 mg./day. On April 22 his blood and bone-marrow were aplastic (Figs. 1 and 2). On April 23 he was given an intravenous injection of 2.000 ml. of a mixture of blood and bone-marrow taken in equal parts from six donors: the patient's father (H), three brothers (D, M, and P), his mother (G), and his sister (F).

TABLE I.—*Erythrocyte Phenotypes of the Host and of the Donors*

Host	B.B.:	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+)

TABLE II.—*Serum Phenotypes of the Host and of the Donors*

Host:	B.B.:	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+)

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†A detailed report of this case will be published later.

The histocompatibility relations were determined on the basis of the erythrocyte phenotypes (Table I), the serum phenotypes (Table II), the leucocyte antigens (Dr. J. Dausset) (Table III), and the histocompatibility test already recommended by us[21 29]; on the basis of the results of this test the donors were classified in the order of closeness to the recipient as follows: P and H, M and F, D and G.

Myeloid restoration was indicated by the reappearance of reticulocytes in the blood on April 25. It progressed in the first 10 days of May (Figs. 1 and 2). During the period of aplasia the patient was nursed in a special aseptic ward and received platelet transfusions (Fig. 1).

During the first few days of May there appeared a secondary syndrome which showed great similarity to that observed in the various species of animals[1 3 7 10 12 32 33 34] and which was characterized by a rapidly progressive loss of weight, digestive disorders (diarrhoea, nausea, and vomiting), desquamative erythrodermia (Fig. 3) (characterized histologically by a dermal infiltration with mononuclear cells, a mild acanthosis, and the

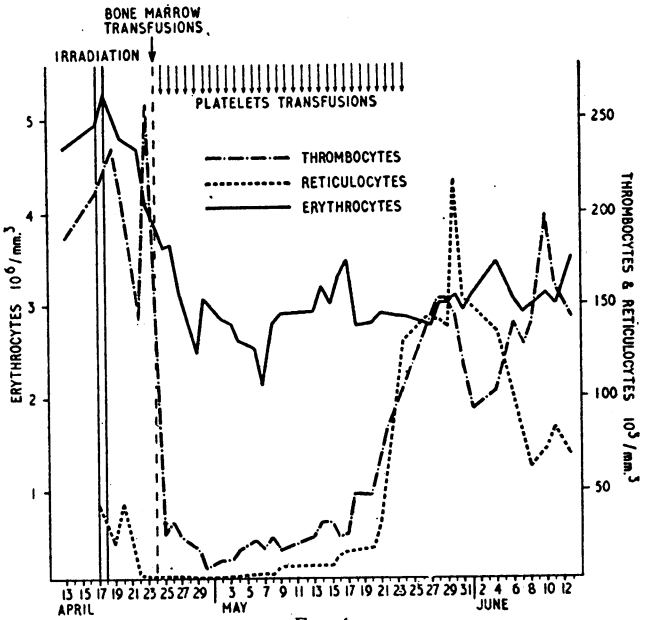

FIG. 1

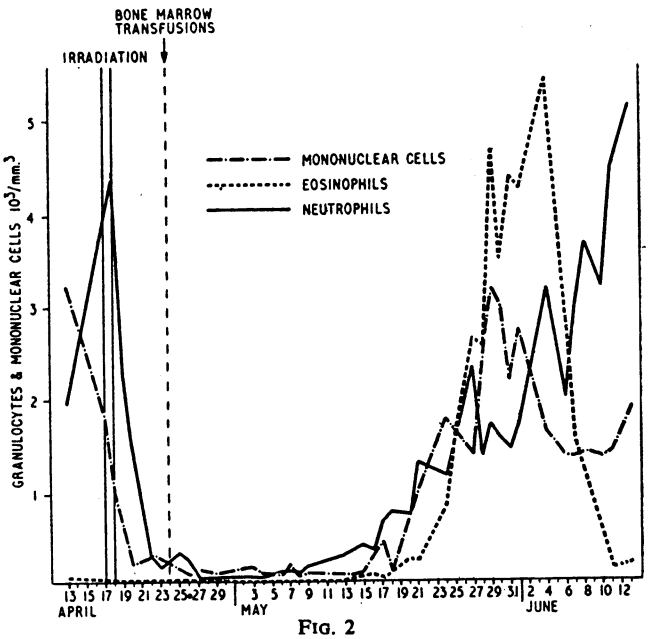

FIG. 2

FIGS. 1 and 2.—Evolution of blood cells during periods of aplasia and restoration.

presence in the epithelium of many eosinophil epithelial cells with pyknotic nuclei and of some basal cells with vacuoles), and further by hepatomegaly with alteration of the concentration of various serum enzymes (Table IV), by micropolyadenopathy (with the lymph nodes characterized histologically and cytologically by absence of lymphoid nodules, lymphocytic

TABLE III.—*Comparative Studies of the Leucocyte Antigens of the Various Donors and of the Host (B.B.) with Sera Containing Leucocyto-agglutinins*

	Corre-spondences		Differences			Corre-spondences		Differences	
B.B. H.B.	+ +	− −	+ −	− +	B.B. D.B.	+ +	− −	+ −	− +
	9	26	4	9		6	25	7	10
	35 times corresponding		13 times different			31 times corresponding		17 times different	
B.B. M.B.	+ +	− −	+ −	− +	B.B. P.B.	+ +	− −	+ −	− +
	7	20	6	15		10	28	3	7
	27 times corresponding		21 times different			38 times corresponding		10 times different	
B.B. G.B.	+ +	− −	+ −	− +	B.B. F.B.	+ +	− −	+ −	− +
	6	27	7	8		11	26	2	9
	33 times corresponding		15 times different			37 times corresponding		11 times different	

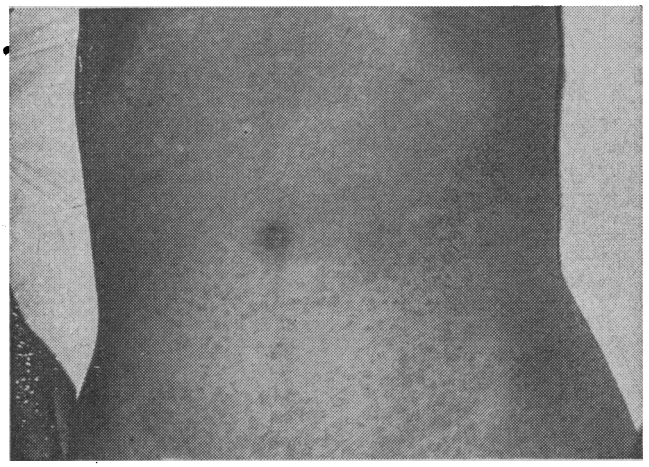

FIG. 3.—Macroscopical aspects of skin erythrodermia

TABLE IV.—*Serum Levels of Various Enzymes*

Date	L.D.H.	M.D.H.	G.O.T.	G.P.T.	Aldolase	O.C.T.
22/4/63	210	115	12	6		
13/5/63	360	90	55	34	0·25	0·33
20/5/63	400	76	10	46	0·24	1·21
29/5/63	*600*	113	**310**	**92**	**0·75**	**2·10**
13/6/63	*550*	115	**220**	**73**	0·35	**1·4**
14/6/63	340	152	**164**	38	*0·48*	**0·69**
18/6/63	295		38	46	0·07	

For L.D.H. (lactic acid dehydrogenase) and M.D.H. (malic acid dehydrogenase) the findings are stated in the Wroblewski spectrophotometric units per ml. of serum and per minute. For the two transaminases, G.O.T. and G.P.T., the results are listed in Frankel units, also per ml. of serum per minute. For aldolase the findings are given in Meyerhof units. For O.C.T. (ornithine-carbamyltransferase) the findings are given in micromoles of $N\text{-}NH_3$ formed per ml. of serum per 24 hours at 40° C.

aplasia, and proliferation of the histiocytes with hyperbasophil cytoplasm of the type described for the graft-versus-host reaction[4]), and, finally, by miliary tuberculosis, by an eosinophilia of more than 50% of the blood cells, and by a decrease of the levels of various serum immunoglobulins (γ, β-2A, and β-2M). The patient was treated symptomatically with isoniazid, streptomycin, Δ-1 cortisone (in doses of 10–25 mg.), and ε-aminocaproic acid. On July 6 the principal signs of the secondary syndrome had disappeared. The patient began to regain weight and was discharged from hospital.

DETERMINATION OF THE BONE-MARROW GRAFT AND THE SPECIFIC TOLERANCE DUE TO THE CHIMERISM

Several examinations have been carried out in an attempt to determine to what extent the haematopoietic restoration is due to the "taking" of the transfused cells.

(*a*) The study, after culturing, of the chromosomes of the circulating leucocytes failed to reveal any radiation damage of the type observed in a patient irradiated with the same dosage[31] and treated with an isogenic bone marrow transfusion (which leads only to transient taking and allows the autogenous restoration to occur). This absence of radiation damage suggests that in the present case the blood-cell population consisted entirely of non irradiated—that is, foreign—blood cells.

(*b*) The study of the erythrocyte phenotype presented by the patient eight months after treatment showed that it was precisely that of the donor P: nearly 100% of the red cells were agglutinated by the anti-s serum and by the anti-Le (a+) serum; no red cells were agglutinated by the anti-c serum or by the serum anti-s. Moreover, the patient also produced a few female cells, as could be demonstrated by the study of the sex chromatin of the leucocytes: from one examination to another, 0 to 4% of the polynuclear cells presented drumsticks; 7 to 41% of the mononuclear cells presented heterochromatic lobules; and 3% of the mitoses presented a female chromosome formula. In other words, the grafting had essentially occurred on the basis of the cells of donor P, and only to a very slight extent on the basis of the cells of one of the female donors.

(*c*) The Inv group presented by the patient eight months after treatment was: Inv (1−a−b+). One can deduce from that change that the host produces γ-globulins and eventually β-2A and β-2M globulins of the donor(s) type.

(*d*) The results of the study of the tolerance connected with the chimerism tend to confirm these conclusions. In fact, on June 6, 1963, the patient was grafted with skin fragments from all the bone-marrow donors and with a fragment from his own skin. Unfortunately the fragment obtained from one of the female donors, F (the patient's sister), was torn off with a bandage. The development of the other grafts was very interesting: the graft from D was rejected on the 15th day, that from M on the 24th day, that from G on the 28th day, and that from H only on the 60th day. The autogenous graft and the graft from P were still in perfect condition at the time of writing (Fig. 4), proving that there existed a specific tolerance for

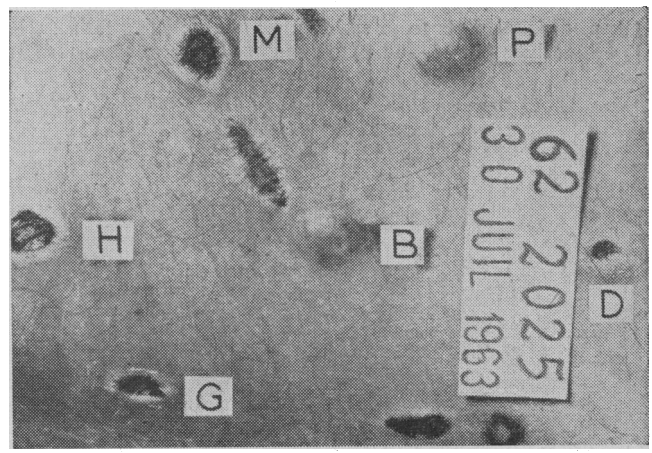

FIG. 4.—Aspects of skin grafts from various donors four months after transplantation. Tolerance of the autogenous and of P grafts (the aspect is the same after eight months).

P which was in all probability due to the haematopoietic chimerism.

(*e*) It will be noted that donor P, whose cells have repopulated the bone-marrow of the host, had been classified as closest by the histocompatibility test[21 29] and by the study of the leucocyte antigens.

CONCLUSIONS AND PERSPECTIVES

This case is the first example in a man of haematopoietic artificially induced chimerism of long duration: at the time of writing it had already lasted longer than eight months, following a conditioning that had been no different from that which had made possible similar grafting in various species of animals.[6 16]

In the light of the above-mentioned experiments in mice,[8] the fact that the patient had had no previous blood transfusions was regarded as particularly important. The use of several donors eliminated a number of difficulties: it made it possible to administer a large number of cells, and it allowed the organism to select the most suitable donor. The histocompatibility test which we have proposed[21 29] and the study of the leucocyte antigens have also proved to yield information that is of value in the selection of donors.

It is still too early to draw any conclusions concerning the antileukaemic effect of this treatment: it must be remembered that what is sought is the combination of the cytodestructive action of the irradiation and an immunological antileukaemic effect brought about by the graft-versus-host reaction; if we postulate a subject A with leukaemic cells *a*, in whom the disease may be due to a virus α, and who is grafted with bone-marrow B, it has been demonstrated that an antileukaemic effect may result from the reaction of the graft B to the *a* cells[2 17 22 24 25 30] and, in the case of a virus leukaemia, to the oncogenic virus α.[18] In any case, the fact that our patient, previously in a phase of relapse with resistance to most forms of treatment, had at the time of writing had eight months of complete remission, constitutes in itself a remarkable result.

This case is also interesting in that it has demonstrated the existence of a specific tolerance connected with the chimerism in man: now that the experiments with renal transplantation after sublethal irradiation or attempts at conditioning with chemical antimitotics have produced disappointing results on the whole,[5 9 11 13 14] it gives new hope to find that the only method that makes it possible to obtain a protracted specific tolerance in the animal[15] can be carried out in man as well.

Finally, the results obtained confirm that allogenic bone-marrow grafting has its place among the methods of treatment of subjects exposed to radiation accidentally.[23 28]

SUMMARY

A patient with leukaemia in the progressive phase, who showed resistance to most chemotherapeutic products, was subjected to whole-body irradiation with a dose of 800 rad, preceded by four days' administration of methyl-nitro-imidazolyl-mercaptopurine. Bone-marrow from six donors was transfused. After eight months the patient's blood was completely repopulated with red cells which showed the antigenic characteristics of one of the donors; he produced γ-globulins of the donor(s) type; the patient also presented a specific tolerance for a skin graft from this donor. A severe secondary syndrome complicated this grafting, but it could be controlled, and at the time of writing the patient was in good condition, with the leukaemia in complete remission.

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REFERENCES

1 Barnes, D. W. H., Ford, C. E., Ibery, P. L. T., Koller, P. C., and Loutit, J. F., *J. cell. comp. Physiol.*, 1957, Suppl. 50, p. 123.
2 —— and Loutit, J. F., *Brit. J. Haematol.*, 1957, **3**, 241.
3 Bekkum, D. W. Van, Vos, O., and Weyzen, W. W. H., *J. nat. Cancer Inst.*, 1959, **23**, 75.
4 Binet, J. L., and Mathé, G., *Ann. N.Y. Acad. Sci.*, 1962, **99**, 426
5 Calne, R. Y., *Renal Transplantation*, 1963. Arnold, London.
6 Cole, L. J., and Alpen, E. L., *Blood*, 1963, **21**, 373.
7 Congdon, C. C., and Urso, I. S., *Radiat. Res.*, 1957, **7**, 310.
8 Da Costa, H., Amiel, J. L., and Mathé, G., Inhibition de la greffe de moelle osseuse allogénique par des transfusions de sang antérieures à l'irradiation, *Vox Sang. (Basel)*. In press.
9 Dempster, W. J., personal communication.
10 Ferrebee, J. W., Lochte, H. L., Jaretzki, A., Sahler, O. D., and Thomas, E. D., *Surgery*, 1958, **43**, 516.
11 Hamburger, J., Vaysse, J., Crosnier, J., Auvert, J., Lalanne, C. M., and Dormont, J., *Rev. franç. Étud. clin. biol.*, 1962, **7**, 20.
12 Ilbery, P. L. T., Koller, P. C., and Loutit, J. F., *J. nat. Cancer Inst.*, 1958, **20**, 1051.
13 Küss, R., Legrain, M., Mathé, G., Nedey, R., and Camey, M., *Postgrad. med. J.*, 1962, **38**, 528.
14 —— —— —— —— Tubiana, M., Lalanne, C. M., Schwarzenberg, L., Larrieu, M. J., Maisonnet, M., Basset, F., and Delaveau, P., *Rev. franç. Étud. clin. biol.*, 1962, **7**, 1028.
15 Main, J. M., and Prehn, R. T., *J. nat. Cancer Inst.*, 1955, **15**, 1023.
16 Mathé, G., and Amiel, J. L., *La greffe: Aspects biologiques et cliniques*, 1 vol., 1962. Masson, Paris.
17 —— —— and Bernard, J., *Bull. Ass. franç. Cancer*, 1960, **47** 331.
18 —— —— and Friend, Ch, ibid., 1962, **49**, 416.
19 —— —— Matsukura, M., and Méry, A. M., *C.R. Acad. Sci (Paris)*, 1962, **255**, 3480.
20 —— —— —— —— —— Restoration of the Haematopoietic Function in the Irradiated Mouse, with Use of Allogenic Bone-marrow Grafts from Several Donors of Different Strains, *Brit. J. Haemat.* In press.
21 —— —— and Niemetz, J., *Rev. franç. Étud. clin. biol.*, 1961, **6**, 684.
22 —— —— —— *C.R. Acad. Sci. (Paris)*, 1962, **254**, 3603.
23 —— —— and Schwarzenberg, L., *N.Y. Acad. Sci.* In press.
24 —— and Bernard, J., *Bull. Ass. franç. Cancer*, 1958, **45**, 289.
25 —— —— *Rev. franç. Étud. clin. biol.*, 1959, **4**, 442.
26 —— —— Schwarzenberg, L., Larrieu, M. J., Lalanne, C. M., Dutreix, A., Denoix, P. F., Surmont, J., Schwarzmann, V., and Céoara, B., ibid., 1959, **4**, 675.
27 —— —— Vries, M. J. de, Schwarzenberg, L., Larrieu, M. J., Lalanne, C. M., Dutreix, A., Amiel, J. L., and Surmont, J., *Rev. Hémat.*, 1960, **15**, 115.
28 —— Jammet, H., Pendic, B., Schwarzenberg, L., Duplan, J. F., Maupin, B., Latarget, R., Larrieu, M. J., Kalic, D., and Djukic, Z., *Rev. franç. Étud. clin. biol.*, 1959, **4**, 226.
29 Matsukura, M., Méry, A. M., Amiel, J. L., and Mathé, G., *Transplantation*, 1963, **1**, 61.
30 Murray, J. E., Merrill, J. P., Dammin, G. J., Dealy, J. B., Alexandre, G. W., and Harrison, J. H., *Ann. Surg.*, 1962, **156**, 337.
31 Papiernik, M., Amiel, J. L., and Mathé, G., *C.R. Acad. Sci. (Paris)*, 1963, **256**, 5232.
32 Porter, K. A., *Brit. J. Cancer*, 1960, **14**, 66.
33 Vries, M. J. de, Crouch, B. G., Putten, L. M. Van, and Bekkum, D. W. Van, *J. nat Cancer Inst.*, 1961, **27**, 67.
34 —— and Vos, O., ibid., 1959, **23**, 1403.