

Bone Marrow Transplantation in Acute Leukemia¹

E. D. THOMAS AND R. B. EPSTEIN

Department of Medicine, University of Washington School of Medicine, Seattle, Washington

SUMMARY

Studies of whole-body irradiation and marrow transplantation as related to the treatment of acute leukemia are reviewed. A discussion is given of current problems of procurement and preservation of marrow, of difficulties in securing allogenic marrow grafts, and of the potential application of cross-circulation to problems in acute leukemia.

Animals given lethal irradiation may be protected from death due to marrow failure by repopulating the marrow spaces with a sufficient number of suitable bone marrow cells (12). A number of investigators have attempted to employ this knowledge in the treatment of leukemia with the intent of destruction of the diseased marrow by irradiation and replacement with normal marrow. This paper will review some of the accomplishments that have resulted from these studies and some of the problems that are current in this field.

STUDIES IN MICE

The feasibility of irradiation and marrow replacement in the treatment of murine leukemia has been explored in several studies. Barnes *et al.* (4) were able to achieve long-term survivors in CBA mice with 151/1 leukemia treated with 1500 r and marrow infusion. Hewitt and Wilson (16) used CBA mice with a different tumor and could not eradicate the leukemia with doses of ⁶⁰Co irradiation of 2400 r. They were able to demonstrate a linear relation between the dose of irradiation and the logarithm of the number of remaining cells.

In evaluating the amount of X-irradiation necessary to sterilize a variety of murine leukemias Burchenal *et al.* (8) found that a range of 2000 to over 5000 r was needed to prevent successful transfer of the leukemia.

Barnes and Loutit (5) found evidence suggesting an advantage of homologous marrow following irradiation of leukemic mice in that the marrow might react immunologically to destroy residual leukemic cells. Mathé (20) explored the utilization of the graft *versus* host reaction of allogenic marrow transplantation as an adjunct to total-body irradiation in the treatment of leukemia. Mice with leukemia, irradiated and given isogenic marrow, invariably died of leukemia. Mice infused with allogenic marrow generally died of secondary disease without morphologic

evidence of leukemia in most cases. Transfer experiments were not done to detect the presence of latent leukemia.

From these studies of murine leukemia it would seem that X-irradiation alone, in doses in which marrow infusion can prevent death, is insufficient to eradicate most leukemias. There is a large variation in the dose of irradiation required for sterilization of different murine leukemias. Allogenic marrow may be beneficial in suppression of leukemia by virtue of the unfavorable environment created by secondary disease.

STUDIES IN MAN

Table 1 summarizes the published data on patients with acute leukemia who have been treated with whole-body irradiation and marrow infusion. Details of most of these cases are provided in other publications (24). Several aspects of these clinical studies deserve comment.

Remission of leukemia by whole-body irradiation without marrow engraftment.—Several patients with acute leukemia in terminal relapse have been given large doses of whole-body irradiation in preparation for a marrow graft. Autogenous regeneration of marrow and remission of the leukemia occurred despite failure of the marrow graft. Examples are the case of a 16-year-old girl with a 6-month remission after 325 r (38) and a 9-month-old infant with a 4-month remission after 800 r (33). Evidently, whole-body irradiation may benefit some patients with acute leukemia after conventional chemotherapy has failed.

Treatment of leukemia by whole-body irradiation with isogenic marrow infusion.—Several sets of identical twins have been studied (3, 34, 37). In each instance 1 twin had acute leukemia, and the other twin was normal insofar as could be determined. Three such patients were irradiated with 800–1600 r. Death from marrow failure was avoided by isogenic marrow infusion. In each instance a remission was achieved, but the improvement was followed by a discouraging early return of leukemia. These studies have shown that man can survive, with a benign clinical course, otherwise lethal doses of irradiation when protected by

¹ Supported by USPHS career program award 1-K6-A1-2425 from the Institute of Allergy and Infectious Diseases and by a USPHS research fellowship.

TABLE 1
SUMMARY OF SOME PUBLISHED OBSERVATIONS ON THE RESULTS OF WHOLE-BODY IRRADIATION AND MARROW INFUSION IN PATIENTS WITH ACUTE LEUKEMIA

AUTHOR	REFERENCE	TYPE OF MARROW			EVIDENCE OF HOMOLOGOUS MARROW ENGRAFTMENT	EVIDENCE OF SECONDARY SYNDROME	RE-MISSION ^a
		Allogenic	Isogenic	Fetal			
Andrews <i>et al.</i>	1	7					2
Arient <i>et al.</i>	2	1					
Atkinson <i>et al.</i>	3		1				1
Beard <i>et al.</i>	6	1					
Clinicopathologic Conference	27	1					
Haurani <i>et al.</i>	15	9					1
King	18	1	1				1
Kurnick	19		2				
Mathé <i>et al.</i>	20 ^b	10			7	7	3
McGovern <i>et al.</i>	23	2	2				1
Pegg <i>et al.</i>	25	2					
Thomas and Ferrebee	31	15	4	3 ^c	1		5
		—	—	—	—	—	—
Total		46	10	3	6	5	14

^a Excluded are patients dying of consequences of irradiation even though there was no evidence of leukemia at autopsy.

^b See footnote 2.

^c Received homologous marrow in addition.

isogenic marrow. However, these doses do not eradicate leukemia, perhaps because some of the leukemic cells escape destruction or perhaps because the isogenic cells are also susceptible to a causative agent. For these same reasons, it seems unlikely that the patient's own marrow, stored during remission of acute leukemia, will be helpful in securing remissions of useful duration following irradiation or chemotherapy (19, 23).

Treatment of leukemia by whole-body irradiation with allogenic marrow infusion.—Table 1 indicates that a number of attempts have been made to secure an allogenic marrow graft in man. Most of these efforts have been disappointing. Thomas *et al.* (39) observed a transient allogenic marrow graft in a patient with chronic lymphocytic leukemia. Mathé *et al.* (22) observed a transient marrow graft following accidental radiation exposure. A fatal secondary syndrome was also observed in leukemic patients with allogenic marrow grafts following irradiation (21).

Despite the general lack of success with allogenic marrow grafts in man, 2 cases offer some encouragement. The first is that of a patient with Hodgkin's disease reported by Beilby *et al.* (7). Severe marrow depression was observed after aminochlorambucil therapy. Recovery occurred following an infusion of marrow from the patient's sister, and successful engraftment was indicated by production of donor-type red cells for at least 9 months.

The second, and more encouraging, case is that of a 26-year-old man studied by Mathé *et al.*² The patient had

²Mathé, G., Amiel, J. L., Schwarzenberg, L., Calton, A., Schneider, M., de Vries, M. J., Tubiana, M., La Lanne, C. M., Binet, J. L., Papiernik, M., Seman, G., Matsukura, M., Mery, A. M., Schwarzmann, V., and Flaisler, A. Haemopoietic Chimerism Following Allogenic Bone Marrow Transplantation in Man. Control of Secondary Syndrome. Specific Tolerance Associated with the Chimerism. *Cancer Res.*, 25: 0000-00, 1965.

had acute leukemia for 2 years, controlled by chemotherapy. Shortly after relapse he was given 800 r of whole-body irradiation, followed by infusions of marrow obtained from 6 relatives. A secondary syndrome was observed but subsided after 2 months. Studies of red cell antigens, leukocytes, and skin grafting tests indicated a successful marrow graft, principally from 1 of the donors. The patient showed no evidence of recurrent leukemia after 7 months.

CURRENT PROBLEMS AND PROSPECTS

The problem of marrow preservation and procurement.—The problem of the preservation of marrow cells at low temperature seems to be solved as a practical procedure, although much work remains to be done in this interesting field of biology. The freezing of marrow cells in dimethylsulfoxide has overcome most of the problems with the glycerol technic (10). Deterioration of the frozen marrow with time does not seem to be a problem at temperatures of -80°C and below (32).

Numbers of marrow cells adequate for isogenic grafts can be obtained by multiple marrow aspirations. In our hands the yield from this procedure for a single donor has ranged from 3 to 17×10^9 nucleated cells. For allogenic grafts it is important to administer a larger number of cells: 10×10^9 for the dog (29) and perhaps 40×10^9 for man. A better method of procuring this number of marrow cells from a living donor is urgently needed. Cadaver marrow can be obtained in large quantity (14), but problems in finding suitable donors and in obtaining the marrow within the necessary time limits pose serious obstacles to the practical use of marrow from this source. At present, fetal marrow seems to offer no advantage over adult marrow (30).

TABLE 2

ALLOGENIC MARROW GRAFTS IN THE DOG

All dogs were given 1500 r and an allogenic marrow infusion. The 2nd group of dogs received methotrexate in the first 10 days after irradiation. The 3rd group of dogs also received methotrexate; in this group donors and recipients were matched for 6 red cell antigens.

Treatment	No. of dogs	No. of "takes"	No. of graft rejections	No. living beyond 150 days
Irradiation: random donor and recipient	10	8	5	1
Irradiation and methotrexate: random donor and recipient	10	10	2	4
Irradiation and methotrexate donor and recipient matched for RBC antigens	10	10	3	5

TABLE 3

SURVIVAL OF IRRADIATED DOGS GIVEN INFUSIONS OF ALLOGENIC MARROW OBTAINED FROM 5, 15, AND 20 DONORS (28)

No. of donors	Recipient's kennel no.	Radiation dose (r)	Marrow dose ($\times 10^9$)	Methotrexate	Days survived
5	624	1500	26.4	Yes	25
	626	1500	26.4	Yes	29
	545	1500	26.4	Yes	45
15	913	1500	52.0	Yes	79
	911	1500	52.0	No	15
20	910	1500	74.0	Yes	37
	914	1500	74.0	Yes	38
	909	1500	74.0	No	12
	912	1500	74.0	No	13

The problem of securing permanent allogenic marrow grafts.—Most of the information now available relative to allogenic marrow grafts was derived from rodents, principally inbred mice. Allogenic marrow grafts in outbred species—dog (29), monkey (41) and man—have been quite difficult to secure. In this regard, the last 2 years' experience with dogs in Cooperstown has been encouraging in that some 40–50% of irradiated animals given allogenic marrow have survived to become long-term radiation chimeras (35, 36). These results are summarized in Table 2. Success in these animals has been associated with a large dose of irradiation (1500 r) and a large dose of marrow (greater than 9×10^9 nucleated cells). The critical factors seemed to be (a) the addition of an immunosuppressive drug to the treatment program and (b) in the instance of success, a fortunate selection of donor and recipient of reasonable histocompatibility.

The immunosuppressive drug, methotrexate, reduced the incidence of marrow graft rejection and reduced the incidence and severity of secondary disease. Selection of donor and recipient of reasonable histocompatibility is not yet a practical procedure. Matching of red cell antigens

has offered no improvement (36). Other histocompatibility typing procedures are being studied in the dog (17). The current success rate of 40–50% in the dog suggests that the number of major histocompatibility groups may be relatively small.

Mathé's exciting case, outlined above, illustrates these points. Immunosuppressive therapy was added to the radiation treatment. The use of 6 donors permitted an *in vivo* selection of a single donor whose marrow was principally responsible for repopulating the marrow spaces. The desirability of selecting this donor prior to the fact of grafting is evident.

The use of multiple donors may not always succeed (28). Table 3 shows the results of some preliminary experiments with multiple donors used in studies with the dog. Graft *versus* graft reactions with associated immunologic death may account for some of these failures.

The potential application of cross-circulation to problems in acute leukemia.—Cross-circulation has long been recognized as a potentially useful procedure in studying the transfer of both humoral and cellular factors between two individuals (13). In the dog, it has been shown that cross-circulation can maintain an effective leukocyte level when 1 member of the pair has received lethal radiation (40). Stored peripheral blood leukocytes have been shown to contain the cells necessary for isogenic marrow repopulation following lethal irradiation (11). Similarly, cross-circulation has resulted in allogenic marrow regeneration in the dog after irradiation.³

The indwelling silastic-Teflon cannulae developed by Quinton *et al.* (26) for use in hemodialysis have solved most of the technical problems associated with cross-circulation in man. In Seattle 2 pairs of patients have been subjected to cross-circulation on a total of 15 occasions without ill effect. In the 1st pair a patient with hepatic coma following acute liver failure demonstrated a dramatic recovery after cross-circulation for 2 periods of 11 and 36 hr (9). In the 2nd pair, a patient with acute marrow failure received a total of 700×10^9 leukocytes over a 13-day period with 2 hr of cross-circulation daily (9).

The potential usefulness of cross-circulation in the irradiated patient with leukemia in tiding him over the consequences of marrow aplasia, in facilitating an allogenic marrow implantation, and in providing an immunologic environment unfavorable to the tumor remain to be studied.

REFERENCES

- Andrews, G. A., Sitterson, B. W., Kretchmar, A. L., and Tanida, R. Studies on Total-Body Irradiation and Attempted Marrow Transplantation in Acute Leukemia. *Acta Haematol.*, 26: 129–53, 1961.
- Arient, M., Skala, E., Polmesil, M., Pala, F., and Dufek, V. On the Treatment of Acute Leukemia by Massive Whole Body Irradiation Combined with Subsequent Bone Marrow Transfusion—A Case Report. *Neoplasma*, 7: 295–304, 1960.
- Atkinson, J. B., Mahoney, F. J., Schwartz, I. R., and Hesch, J. A. Therapy of Acute Leukemia by Whole-Body Irradiation

³ Collins, J. A., Hechtman, H. B., Theobald, T. G., Blumenstock, D. A., Thomas, E. D., and Ferrebee, G. W. Treatment by Transfusion of Severe General Radiation Injury in Dogs. *Transfusion* (in press.)

- and Bone Marrow Transplantation from an Identical Normal Twin. *Blood*, *14*: 228-34, 1959.
4. Barnes, D. W. H., Corp, M. J., Loutit, J. F., and Neal, F. E. Treatment of Murine Leukemia with X-ray and Homologous Bone Marrow. Preliminary Communication. *Brit. Med. J.*, *2*: 626-27, 1956.
 5. Barnes, D. W. H., and Loutit, J. F. Treatment of Murine Leukaemia with X-rays and Homologous Bone Marrow: II. *Brit. J. Haematol.*, *3*: 241-52, 1957.
 6. Beard, A. G., Barnhard, H. J., Ross, S. W., and Conlin, F. D. Acute Leukemia Treated by Irradiation and Marrow Transplant. *J. Pediat.*, *55*: 42-50, 1959.
 7. Beilby, J. O. W., Cade, I. S., Jelliffe, A. M., Parkin, D. M., and Stewart, J. W. Prolonged Survival of Bone-Marrow Graft Resulting in a Blood-Group Chimera. *Brit. Med. J.*, *1*: 96-99, 1960.
 8. Burchenal, J. H., Oettgen, H. F., Holmberg, E. A. D., Hemphill, S. C., and Reppert, J. A. Effect of Total-Body Irradiation on the Transplantability of Mouse Leukemias. *Cancer Res.*, *20*: 425-30, 1960.
 9. Burnell, J. M., Thomas, E. D., Ansell, G. S., Cross, H. E., Dillard, D. H., Epstein, R. B., Eschbach, J. W., Jr., Hogan, R., Hutchings, R. H., Motulsky, A., Ormsby, J. W., Paffenbarger, P., Scribner, B. H., and Volwiler, W. Observations on Cross Circulation in Man. *Am. J. Med.*, *38*: 832-41, 1965.
 10. Cavins, J. A., Kasakura, S., Thomas, E. D., and Ferrebee, J. W. Recovery of Lethally Irradiated Dogs Following Infusion of Autologous Marrow Stored at Low Temperature in Dimethyl-Sulfoxide. *Blood*, *20*: 730-34, 1962.
 11. Cavins, J. A., Scheer, S. C., Thomas, E. D., and Ferrebee, J. W. The Recovery of Lethally Irradiated Dogs Given Infusions of Autologous Leukocytes Preserved at -80°C . *Ibid.*, *23*: 38-43, 1964.
 12. Congdon, C. C. Experimental Treatment of Total-body Irradiation Injury: A Brief Review. *Ibid.*, *12*: 746-54, 1957.
 13. Egdahl, R. H. Physiological Basis of Uncontrolled Cross-Circulation in Dogs. *Am. J. Physiol.*, *182*: 454-58, 1955.
 14. Ferrebee, J. W., Atkins, L., Lochte, H. L. Jr., McFarland, R. B., Jones, A. R., Dammin, G. J., and Thomas, E. D. The Collection, Storage and Preparation of Viable Cadaver Marrow for Intravenous Use. *Blood*, *14*: 140-47, 1959.
 15. Haurani, F. I., Repplinger, E., and Tocantins, L. M. Attempts at Transplantation of Human Bone Marrow in Patients with Acute Leukemia and Other Marrow Depletion Disorders. *Am. J. Med.*, *28*: 794-806, 1960.
 16. Hewitt, H. B., and Wilson, C. W. A Survival Curve for Mammalian Leukemia Cells Irradiated in Vivo (Implications for the Treatment of Mouse Leukemia by Whole-Body Irradiation). *Brit. J. Cancer*, *13*: 69-75, 1959.
 17. Kasakura, S., Thomas, E. D., and Ferrebee, G. W. Leukocytotoxic Isoantibodies in the Dog. *Transplantation*, *2*: 274-80, 1964.
 18. King, R. E. Use of Total-Body Radiation in the Treatment of Far-Advanced Malignancies. *J. Am. Med. Assoc.*, *177*: 610-13, 1961.
 19. Kurnick, N. B. Autologous and Isologous Bone Marrow Storage and Infusion in the Treatment of Myelo-Suppression. *Transfusion*, *2*: 178-87, 1962.
 20. Mathé, G. Secondary Syndrome: A Stumbling Block in the Treatment of Leukemia by Whole-Body Irradiation and Transfusion of Allogenic Hemopoietic Cells. *In: Diagnosis and Treatment of Acute Radiation Injury*, pp. 191-223, Geneva: World Health Organization, 1961.
 21. Mathé, G., Bernard, J., de Vries, M. J., Schwarzenberg, L., Larrieu, M. J., LaLanne, C. M., Dutreix, A., Amiel, J. L., and Surmont, J. Nouveaux essais de greffe de moelle osseuse homologue après irradiation totale chez des enfants atteints de leucémie aigue en remission. Le problème du syndrome secondaire chez l'homme. *Rev. Hematol.*, *15*: 115-61, 1960.
 22. Mathé, G., Jammot, H., Pendic, B., Schwarzenberg, L., Duplan, J. F., Maupin, B., Latarjet, R., Larrieu, M. J., Kalic, D., and Djukic, Z. Transfusions et greffes de moelle osseuse homologue chez des humains irradiés à haute dose accidentelle. *Rev. Franc. Etudes Clin. Biol.*, *4*: 226-38, 1959.
 23. McGovern, J. J., Russell, P. S., Atkins, L., and Webster, E. W. Treatment of Terminal Leukemic Relapse by Total-Body Irradiation and Intravenous Infusion of Stored Autologous Bone Marrow Obtained during Remission. *New Engl. J. Med.*, *260*: 675-83, 1959.
 24. Miller, D. G., and Diamond, H. D. The Biological Basis and Clinical Application of Bone Marrow Transplantation. *Med. Clin. N. Am.*, *45*: 711-31, 1961.
 25. Pegg, D. E., Humble, J. G., and Newton, K. A. The Clinical Application of Bone Marrow Grafting. *Brit. J. Cancer*, *16*: 417-35, 1962.
 26. Quinton, W. E., Dillard, D. H., and Scribner, B. H. Eight Months' Experience with Silastic-Teflon Bypass Cannulas. *Trans. Am. Soc. Artificial Internal Organs*, *8*: 236-45, 1962.
 27. Recant, L., and Hartroft, W. S. (eds.). Total Body Irradiation in Acute Monocytic Leukemia. (Clinico-Pathologic Conference.) *Am. J. Med.*, *25*: 430-41, 1958.
 28. Thomas, E. D. Observations on Supra-lethal Whole Body Irradiation and Marrow Transplantation in Man and Dog. *Am. N. Y. Acad. Sci.*, *114*: 393-96, 1964.
 29. Thomas, E. D., Collins, J. A., Herman, E. C., Jr., and Ferrebee, J. W. Marrow Transplants in Lethally Irradiated Dogs Given Methotrexate. *Blood*, *19*: 217-28, 1962.
 30. Thomas, E. D., Collins, J. A., Kasakura, S., and Ferrebee, J. W. Lethally Irradiated Dogs Given Infusions of Fetal and Adult Hemopoietic Tissue. *Transplantation*, *1*: 514-20, 1963.
 31. Thomas, E. D., and Ferrebee, J. W. Irradiation and Marrow Transplantation. *Studies in Cooperstown. Lancet*, *1*: 1289-90, 1960.
 32. ———. Prolonged Storage of Marrow and Its Use in the Treatment of Radiation Injury. *Transfusion*, *2*: 115-17, 1962.
 33. Thomas, E. D., Herman, E. C., Jr., Cannon, J. H., Sahler, O. D., Ferrebee, J. W., Kay, H. E. M., and Constantoulakis, M. Autogenous Recovery of Marrow Function. *Arch. Internal Med.*, *107*: 395-400, 1961.
 34. Thomas, E. D., Herman, E. C. Jr., Greenough, W. B., III, Hager, E. B., Cannon, J. H., Sahler, O. D., and Ferrebee, J. W. Irradiation and Marrow Infusion in Leukemia. *Ibid.*, *107*: 829-45, 1961.
 35. Thomas, E. D., Kasakura, S., Cavins, J. A., and Ferrebee, J. W. Marrow Transplants in Lethally Irradiated Dogs. The Effect of Methotrexate on Survival of the Host and the Homograft. *Transplantation*, *1*: 571-74, 1963.
 36. Thomas, E. D., Kasakura, S., Cavins, J. A., Swisher, S. N., and Ferrebee, J. W. Significance of Blood Groups in Homotransplantation of Marrow in the Dog. *Ann. N. Y. Acad. Sci.*, *120*: 362-66, 1964.
 37. Thomas, E. D., Lochte, H. L., Jr., Cannon, J. H., Sahler, O. D., and Ferrebee, J. W. Supralethal Whole-Body Irradiation and Isologous Marrow Transplantation in Man. *J. Clin. Invest.*, *38*: 1709-16, 1959.
 38. Thomas, E. D., Lochte, H. L., Jr., and Ferrebee, J. W. Irradiation of the Entire Body and Marrow Transplantation: Some Observations and Comments. *Blood*, *14*: 1-23, 1959.
 39. Thomas, E. D., Lochte, H. L., Jr., Lu, W. C., and Ferrebee, J. W. Intravenous Infusion of Bone Marrow in Patients Receiving Radiation and Chemotherapy. *New Engl. J. Med.*, *267*: 491-96, 1957.
 40. Thomas, E. D., Plain, G. L., Cavins, J. A., and Blumenstock, D. A. Leukokinetics in Dogs Given Supralethal Irradiation with or without a Graft of Marrow. *Ann. N. Y. Acad. Sci.*, *113*: 1020-32, 1964.
 41. Van Bekkum, D. W., Crouch, B. G., and Overman, R. R. (eds.). Bone Marrow Therapy and Protection in Irradiated Primates. Rijswijk: Krips, 1962.