BONE MARROW TRANSPLANTATION — PAST, PRESENT AND FUTURE

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by

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During my time in medical school in the 1940's it was well known that grafts of organs between individuals of different genetic origin always result in rejection. Burnett's hypothesis and Medawar's experiments began to shed some light on transplantation immunology and the possibility of tolerante (Nobel Laureates, 1960). Inbred mice and human identical twins appeared to accept grafts from each other. Dr. Murray and his colleagues were the first to carry out a kidney transplant between human identical twins, a feat of heroic proportions at the time but now almost routine. Grafting of bone marrow was an even more remote possibility since marrow cells could not be sewn into place.

In 1939, Osgood et al.(l) infused a few ml of marrow into patients with aplastic anemia without benefit. Rekers et al.(2) worked in the classified laboratories of the Atomic Energy Commission in Rochester, N. Y. attempting to reconstitute marrow function in irradiated dogs by marrow infusions. These studies were published in 1950. In retrospect, these experiments apparently failed because the irradiation exposures, although lethal, were not great enough to produce the immunosuppression necessary for allogeneic engraftment.

In 1949, Jacobson et al.(3) found that mice could be protected from otherwise lethal irradiation by shielding the spleen with lead. It was initially thought that the protective effect was due to humoral stimulants released by the protected spleen. Soon thereafter Lorenz et al.(4) showed that a similar protection could be achieved by an intravenous infusion of marrow from a mouse of the same strain. At that time I was intrigued by these experiments because of my own work with stimulating factors released by irradiated yeast (5) and by in vitro studies of marrow metabolism (6,7).

In 1955, Main and Prehn published a paper showing that mice protected from lethal irradiation by marrow infusion would accept a skin graft from the donor strain, an observation strongly suggesting that a transfer of living donor cells had occurred to account for the apparent tolerance (8). Also in 1955, Ford et al.(9) used cytogenetics to show the presence of donor cells in irradiated mice protected by a marow infusion. In the summer of 1955 I

moved to the Mary Imogene Bassett Hospital in Cooperstown, N.Y. where I found that Dr. Joseph Ferrebee had been following these and related experiments very closely. We decided to begin studies of marrow grafting in outbred species, especially the canine model, and to begin cautious exploration of marrow infusion in human patients in need of a marrow graft because of disease or its treatment.

In 1957, we published our first paper on human marrow grafting which we called "intravenous infusion" because only one patient had a transient graft which hardly constituted true marrow transplantation (10). We learned two things from those studies: 1) Large quantities of human marrow could be infused intravenously without harm when properly prepared; and 2) allogeneic marrow grafting in our species would be very difficult. Because of concern about irradiation exposure our early funding came through the Atomic Energy Commission. In the 1957 paper we stated "The studies presented here show that human bone marrow can be collected and stored in significant quantities and can be administered with safety. After administration it may grow even under disadvantageous competitive circumstances in completely irradiated hosts afflicted with marrow neoplasia. In an atomic age, with reactor accidents not to mention stupidities with bombs, somebody is going to get more radiation than is good for him. If infusion of marrow can induce recovery in a mouse or monkey after lethal radiation, one had best be prepared with this form of treatment in man. The leukemic patient who needs radiation and bone marrow and the uremic patient who needs a spare kidney are people who deserve immediate consideration. From helping them one will be preparing for the atomic disaster of tomorrow and it is high time one did." The reference to a spare kidney was based on the possibility that a marrow graft might be necessary to permit permanent acceptance of a kidney from the same donor. Immunosuppressive drugs for organ grafting were unknown at that time.

Allogeneic marrow grafting continued to be unsuccessful, but we did have the opportunity to carry out marrow grafts between a few sets of identical twins. Our first two patients, reported in 1959, with refractory leukemia who had an identical twin were given supralethal irradiation and a syngeneic marrow infusion (11). Their prompt hematologic recovery and well being demonstrated that an intravenous infusion of marrow could protect against lethal irradiation. The recurrence of their leukemia in a few months prompted our speculation about how marrow grafting might cure leukemia as follows: "Evidently something more than radiation is needed to eradicate leukemia. Two possible approaches are suggested. First, one may transplant homologous marrow after lethal irradiation and depend on the homologous marrow to provide an immunologic environment unsuitable for survival of the leukemia (Barnes et al. 1956). This approach has apparently eradicated leukemia in some mice (Barnes et al., 1957, Mathé and Bernard, 1958). However, these mice subsequently have a high incidence of death from delayed foreign marrow disease due to a reaction of the graft against the host. Whether delayed foreign marrow disease will be either

serious or useful in man and whether it can be controlled by clinical supportive measures available are questions currently being studied." "The second approach to the problem of eradicating leukemia lies in the observation that with chemotherapy and x-ray, the cure rate of transplantable leukemia in the mouse is an inverse function of the number of cells present - the smaller the number of leukemic cells, the greater the possibility of cure (Burchenal et al., 1951, Mathé et al., 1959). This suggests that the patient in remission, with a relatively small mass of leukemic cells, is an advantageous subject for radiation. It further suggests that chemotherapeutic agents may be more effective if administered during an immediate postradiation period when the number of leukemic cells is relatively small." Unfortunately, 15 years were to go by before we were able to carry out marrow grafts for patients with leukemia in remission.

During the late 1950's other investigators attempted allogeneic grafts in human beings. Mathé and his colleagues reported the Yugoslavian radiation accident cases in 1959, several of whom were treated with marrow infusions (12). A retrospective review of these case suggested little benefit (13). Mathé did achieve a durable allogeneic marrow graft in a patient with leukemia,. only to have that patient develop chronic graft-versus-host disease and die of infectious complications (14).

Beginning in 1955, Dr. Joseph Ferrebee, Dr. Harry Lochte, Jr. and I and our colleagues carried out marrow grafting studies in the dog. The dog is a readily available outbred animal often used in transplantation research and amenable to clinical procedures comparable to those used for human patients. When Dr. John Mannick worked with us as a fellow, we found that dogs could be given three times the lethal dose of total body irradiation and recover promptly if given an infusion of their own marrow set aside before irradiation (15). Marrow grafts could be obtained with peripheral blood as well as marrow (16) and the cells responsible for recovery could be frozen and kept for long periods of time (17,18).

However, in the dog as in our human patients marrow from an allogeneic donor almost always resulted either in failure of engraftment or in successful engraftment followed by lethal graft-versus-host disease (19,20). We were encouraged by the fact that an occasional dog, usually with a littermate donor, went through the grafting procedure successfully. The persistence of donor marrow was confirmed by cytogenetic studies when donor and recipient were of opposite sex (21) and many of these dogs proved to live a normal canine life span (22). Evidently it could be done — we just had to find out how.

Delta Uphoff had reported that methotrexate ameliorated graft-versus-host disease in some strains of mice (23). We found methotrexate given post grafting to be of help in reducing the incidence and severity of the graft-versus-host reaction, and a great deal of work went into the study of various methotrexate regimens (20,24). These and other studies in the canine model produced a wealth of information, summarized in 1972 (25). Most importantly, it was clear that a successful allogeneic graft depended upon

close histocompatibility matching between donor and recipient, and we developed techniques for histocompatibility typing in the dog (26). We were finally able to detect DL-A antigens and to show that marrow grafts between matched littermates were almost always successful (27). These studies pointed the way for marrow grafting in man using patients with an HL-A matched sibling donor.

The many failures of allogeneic marrow grafting in human patients caused most investigators to abandon such studies in the 1960's. However, under the impetus of kidney grafting, the knowledge of human histocompatibility antigens progressed rapidly. As we developed our knowledge of DL-A matching, we followed closely the work of Dausset (Nobel Laureate, 1980), van Rood, Payne, Bodmer and Amos in the human, the HL-A system. By 1967, we thought that the time was right to return to allogeneic marrow grafting in humans. Recognizing that the care of patients with advanced leukemia undergoing allogeneic grafts would be difficult, we began to assemble the necessary team. In 1967 we wrote a program project grant application which was funded by the National Cancer Institute in 1968. We began to assemble and train a team of nurses familiar with the care of patients without marrow function and subject to opportunistic infections. In November of 1968 Dr. Robert Good and his colleagues carried out the first marrow transplant from a matched sibling for an infant with an immunological deficiency disease (28). Our team carried out our first transplant using a matched sibling donor for a patient with advanced leukemia in March 1969.

These studies marked the beginning of the "modern" era of human allogeneic marrow grafting. A comprehensive review of the experimental background, the early clinical successes and the deliniation of problems was presented in the New England Journal of Medicine in 1975 (29). As follow-up times increased for patients transplanted for end-stage leukemia, it became apparent that a plateau was developing on a Kaplan-Meier plot of survival so that it became possible to use the term "cure" for these patients (30).

Allogeneic marrow grafts are now carried out at more than 200 centers around the world, and the number of diseases for which marrow grafting may be considered continues to increase. Currently, approximately 5,500 allogeneic and 4,000 autologous marrow transplants are performed annually (Mary Horowitz, International Bone Marrow Transplant Registry, Personal communication). The longest survivors of these otherwise lethal diseases are now 20 years post-grafting. Recent articles summarize the experience of the Seattle team with patients given allogeneic marrow grafts for acute myeloid leukemia (31,32), chronic myeloid leukemia (33,34) and aplastic anemia and thalassemia major (35). Lucarelli et al. (36) have described their extensive experience with thalassemia major.

Among the early problems in allogeneic marrow grafting, perhaps the greatest was immunologic reactivity of the host (graft rejection) and/or of the graft (graft-versus-host disease). Irradiation of the host with "supra-

lethal" exposures was necessary for retention of the marrow graft. Obviously, irradiation could not be used after the graft. The use of methotrexate was mentioned above, but better agents were on the way. Schwartz and Dameshek (37) noted the immunosuppresive properties of 6-mercaptopurine and Hitchings and Elion (Nobel laureates, 1988) developed Immuran. Santos and Owens introduced cyclophosphamide as an immunosuppressive agent for transplantation (38). More recently, cyclosporine has provedinvaluable for organ grafts. Cyclosporine was not superior to methotrexate in our randomized clinical trials of marrow grafter patients, but the regimen of a combination of short methotrexate combined with 6 months of cyclosporine proved effective and is now our standard regimen (39,40). Chronic graft-versus-host disease is severe in a small fraction of patients, but can sometimes be controlled by prolonged corticosteroid therapy (41). T-cell depletion of the marrow graft has resulted in a reduced incidence of graft-versus-host disease but at an increased risk of graft failure or recurrence of malignancy (42). Newer agents are being investigated.

Recurrence of malignant disease following an otherwise successful allogeneic graft continues to be a problem. Efforts to kill a greater fraction of the malignant cells have involved a variety of high dose chemotherapy regimens with or without total body irradiation. Efforts to increase the intensity of the pre-transplant regimen have been limited by life-threatening damage to other organs, most notably the liver and lung (43). The role of biological response modifiers such as interferon is being investigated.

Because of the graft-versus-host reaction and its treatment, patients are profoundly immuno-suppressed following an allogeneic marrow graft and therefore at great risk for all kinds of opportunistic infections (44). Bacterial and some fungal diseases can be controlled by antibiotics, sometimes with the aid of granulocyte transfusions (45). Prophylactic acyclovir has prevented Herpes simplex and zoster infections (46). Pneumonia due to cytomegalovirus (CMV) has been difficult to treat and is a major cause of death (47). For patient and donor pairs serologically negative for CMV, the use of blood products from CMV negative donors has prevented infection (48). The use of prophylactic ganciclovir seems to prevent CMV infection even when donor and/or recipient are CMV positive. Prevention of CMV infection should result in an appreciable increment in long-term survivors of allogeneic marrow grafts.

Only about one-fourth to one-third of patients will have an HL-A identical sibling. Examination of the extended family will identify a non-sibling donor in about 10 percent of patients and results with these donors are comparable to those with an identical sibling (49). With national and international cooperation large panels of volunteer donors whose tissue type is known are now being established. More than 300 transplants using volunteer donors matched to the recipient by computer search have now been carried out. The results using phenotypically matched donors or donors differing by only one HL-A haplotype seem comparable to matched sibling donors (50,51).

In the absence of a suitable matched donor the patient's own marrow may be removed, stored and given back after intensive therapy. The general principles of long-term marrow storage and autologous marrow transplantation have been known for almost 30 years (52). Recently, there has been a striking increase in the use of autologous marrow grafts as more effective measures for destruction of the tumor in the patient have been developed. Methods for destruction of tumor in the marrow graft are being developed. An autologous graft avoids the problems of graft-versus-host disease but may be associated with a greater relapse rate due to the loss of the graft-versus-leukemia effect and the possibility of tumor cells in the stored marrow.

Monoclonal antibodies (Köhler and Milstein, Nobel laureates, 1984) are being used in many ways in marrow grafting. Anti T-cell antibodies for many T-cell epitopes are being used in vitro to remove normal or malignant T-cells from marrow and in vivo to prevent or treat graft-versus-host disease. Monoclonal antibodies coupled with a toxin are being used to treat graft-versus-host disease and, coupled to radioactive isotopes, for selective irradiation exposure of marrow cavities or of tumors so that exposure of the total body to irradiation can be reduced.

Recently, hematopoietic growth factors produced by recombinant molecular biology techniques are being used in marrow grafting. Clinical trials have shown that G-CSF and GM-CSF can accelerate marrow recovery after either allogeneic or autologous marrow grafts. Other growth factors are now entering clinical trial. Biological response modifiers including IL-2, IL-6, cloned T-cells and interferon are being explored for acceleration of marrow graft recovery, for better antibacterial and antifungal effects and greater antitumor effects.

Progress has been made in the identification and purification of the hematopoietic stem cell, long a goal of experimental hematologists. Purified stem cells, free of tumor cells, may be of value in autologous marrow grafting. Retroviral vectors have increased the efficiency of gene transfer and purified stem cells are ideal targets for gene transfer therapy for many diseases. Sustained expression of genes transferred into hematopoietic stem cells has not yet been achieved and the application of gene transfer technology to diseases such as thalassemia major must await much further research.

In summary, marrow grafting has progressed from a highly experimental procedure to being accepted as the preferred form of treatment for a wide variety of diseases at many varying stages of disease. Table 1 shows the approximate 5 year disease-free survival for the most common diseases treated by marrow transplantation. Progress has been slow but steady. Important new developments are showing the way to a further improvement in results so that many more patients with otherwise incurable diseases will have a reasonable chance of long survival and cure.

Finally, it should be noted that marrow grafting could not have reached clinical application without animal research, first in inbred rodents and

then in outbred species, particularly the dog. Application to human patients depended upon developments in many branches of science including understanding of the human histocompatibility system, knowledge of immunosuppressive drugs, blood transfusion technology, especially the ability to transfuse platelets, the creation of a repertoire of broad spectrum antibiotics and the development of effective anticancer chemotherapeutic agents. I echo the sentiments of many previous Nobel laureates when I say that the success we celebrate today was made possible by the work of many others in this and in related fields.

Table I

Diseases	*Survival*
Acute leukemia in relapse	0.10 – 0.30
ALL,first or second remission	0.30 – 0.60
AML, first remission	0.45 – 0.70
CML, chronic phase	0.60 – 0.90
CML, accelerated or blastic phase	0.10 – 0.30
Lymphoma, Hodgkin's disease	
after failure of first line therapy	0.40 – 0.60
after failure of second line therapy	0.10 – 0.30
Immunological deficiency disease	0.50 – 0.90
Aplastic anemia, transfused	0.50 – 0.70
Aplastic anemia, untransfused	0.80 – 0.90
Thalassemia major	
without liver damage	0.85 – 0.95
with liver damage	0.60 – 0.85

Abbreviations:
AML, acute myeloid leukemia
ALL, acute lymphoblastic leukemia
CML, chronic myeloid leukemia

REFERENCES

1. E.E. Osgood, M.C. Riddle and T.J. Mathews, *Ann. Intern. Med.* **13,** 357-367 (1939).
2. P.E. Rekers, M.P. Coulter and S. Warren, *Arch. Surg.* **60,** 635-667 (1950).
3. L.O. Jacobson, E.K. Marks, M.J. Robson, E.O. Gaston and R.E. Zirkle, J. *Lab. Clin. Med.* **34,** 1538 - I543 (1949).
4. E. Lorem, D. Uphoff, T.R. Reid and E. Shelton, *J. Natl. Cancer Inst.* **12,** 197-201 (1951).
5. E.D. Thomas, F.B. Hershey, A.M. Abbate and J.R. Loofbourow, *J. Biol. Chem.* **196,** 575-582 (1952).
6. E.D. Thomas, *Blood* **10,** 600-611 (1955).
7. E.D. Thomas and H.L. Lochte,Jr., *Blood* **12,** 1086- 1095 (1957).
8. J.M. Main and R.T. Prehn, *J. Natl. Cancer Inst.* **15,** 1023- 1029 (1955).
9. C.E. Ford, J.L. Hamerton, D.W.H. Barnes and J.F. Loutit, *Nature* **177,** 452-454 (1956).
10. E.D. Thomas, H.L. Lochte, Jr., W.C. Lu and J.W. Ferrebee, *N. Engl. J. Med.* **257,** 491-496 (1957).

11. E.D. Thomas, H.L. Lochte,Jr., J.H. Cannon, O.D. Sahler and J.W. Ferrebee, *J. Clin. Invest.* **38,** 1709- 1716 (1959).
12. G. Mathé, H. Jammet, B. Pendic, et al., *Rev. Franc. Etudes Clin. et Biol.* IV, 226-238 (1959).
13. G.A. Andrews, *Am. J. Roentgenol. Radium Ther. Nucl. Med.* **93,** 56-74 (1965).
14. G. Mathé, *Diagnostic et Traitement des Radiolesions aigues,* O.M.S., Geneva, p. 197-230 (1964).
15. J.A. Mannick, H.L. Lochte, Jr., C.A. Ashley, E.D. Thomas and J.W. Ferrebee, *Blood* **15,** 255-266 (1960).
16. J.A. Cavins, S.C. Scheer, E.D. Thomas and J.W. Ferrebee, *Blood* **23,** 38-43 (1964).
17. E.D. Thomas and J.W. Ferrebee, *Transfusion* **2,** 115-117 (1962).
18. J.A. Cavins, S. Kasakura, E.D. Thomas and J.W. Ferrebee, *Blood* **20,** 730-734 (1962).
19. E.D. Thomas, C.A. Ashley, H.L. Lochte,Jr., A. Jaretzki,III, O.D. Sahler and J.W. Ferrebee, *Blood* **14,** 720-736 (1959).
20. E.D. Thomas, J.A. Collins, E.C. Herman, Jr., and J.W. Ferrebee, *Blood* **19,** 217-228 (1962).
21. R.B. Epstein and E.D. Thomas, *Transplantation* **5,** 267-272 (1967).
22. E.D. Thomas, G.L. Plain, T.C. Graham and J.W. Ferrebee, *Blood* **23,** 488-493 (1964).
23. D.E. Uphoff, *Proc. Soc. Exp. Biol. Med.* **99,** 651 - 6 5 3 (1958).
24. R. Storb, R.B. Epstein, T.C. Graham and E.D. Thomas, *Transplantation* **9,** 240-246 (1970).
25. R. Storb and E.D. Thomas, in *Proceedings of the Sixth Leukocyte Culture Conference,* Ed.M.R. Schwarz). Academic Press, New York, pp. 805-840 (1972).
26. R.B. Epstein, R. Storb, H. Ragde and E.D. Thomas, *Transplantation* **6,** 45-58 *(1968).*
27. R. Storb, R.H. Rudolph and E.D. Thomas, *J. Clin. Invest.* **50,** 1272- 1275 (1971).
28. R.A. Gatti, H.J. Meuwissen, H.D. Allen, R. Hong and R.A. Good, *Lancet* ii, 1366- 1369 (1968).
29. E.D. Thomas, R. Storb, R.A. Clift, et al., *N. Engl. J. Med.* **292,** 832-843,-895-902 (1975).
30. E.D. Thomas, N. Flout-troy, CD. Buckner, et al., *Leuk. Res.* **1,** 67 - 70 (1977).
31. R.A. Clift, C.D. Buckner, E.D. Thomas, et al., Bone *Marrow Transplantation* **2,** 243-258 (1987).
32. R.A. Clift, C.D. Buckner, F.R. Appelbaum, et al., *Blood, 76:* 1867- 1871 (1990).
33. E.D. Thomas, R.A. Clift, A. Fefer, et al., *Ann. Intern. Med.* **104,** 155- 163 (1986).
.34. E.D. Thomas and R.A. Clift, *Blood* **73,** 861-864 (1989).
35. R. Storb, C. Anasetti, F. Appelbaum, et al., *Seminars in Hematology,* (in press).
36. G. Lucarelli, M. Galimberti, P. Polchi, et al., *N. Engl. J. Med.* **322,** 417-421 (1990).
37. R. Schwartz and W. Dameshek, *Nature* **183,** 1682- 1683 (1959).
38. G.W. Santos and A.H. Owens, Jr., *Transplant. Proc.* **1,** 44-46 (1969).
39. R. Storb, H.J. Deeg, L.D. Fisher, et al., *Blood* **71,** 293-298 (1988).
40. R. Storb, H.J. Deeg, M. Pepe, et al., *Br. J. Haematol.* **72,** 567-572 (1989).
41. K.M. Sullivan, R.P. Witherspoon, R. Storb, et al., *Blood* **72,** 555-561 (1988).
42. P.J. Martin, J.A. Hansen, B. Torok-Storb, et al., *Bone Marrow Transplantation* **3,** 445-456 (1988).
43. S.I. Bearman, F.R. Appelbaum, A. Back, et al., *J. Clin. Oncol.* **7,** 1288- 1294 (1989).
44. R. Witherspoon, D. Noel, R. Storb, H.D. Ochs and E.D. Thomas, *Transplant. Proc.* **10,** 233-235 (1978).

45. C.D. Buckner, R.A. Clift, J.E. Sanders and E.D. Thomas, *Transplant. Proc.* **10,** 255-257 (1978).
46. J.D. Meyers, *Scand. J. Infect. Dis.* Suppl. **47,** 128- 136 (1985).
47. J.D. Meyers, N. Flournoy and E.D. Thomas, *Rev. Infect. Dis.* **4,** 1119- 1132 (1982).
48. R.A. Bowden, M. Sayers, N. Flournoy, et al., *N. Engl. J. Med.* **314,** 1006-1010 (1986).
49. P.G. Beatty, R.A. Clift, E.M. Mickelson, et al., *N. Engl. J. Med.* **313,** 765-771 (1985).
50. J.A. Hansen, C. Anasetti, P.G. Beatty, et al., *Bone Marrow Transplantation* **6,** 108-111 (1990).
51. P.G. Beatty, J.A. Hansen, E.D. Thomas, et al., *Transplantation,* (in press).
52. C.D. Buckner, F.R. Appelbaum and E.D. Thomas, in *Organ Preservation for Transplantation, Chapter 16,* (A.M. Karow, Jr. and D.E. Pegg, Eds.). Marcel Dekker Inc., New York, pp. 355-375 (1981).