Annotation

Journal of Medical Genetics (1970). **7**, 305.

Animal Models for Bone-marrow Transplantation

Bone-marrow transplantation has been tried in Man for many years, but successes, in the form of implantation and long-lasting proliferation of the injected cells, have been few, and it has not yet become established as a routine therapeutic procedure. The feasibility for bone-marrow transplantation and the impetus for contemporary attempts in Man came from animal studies. The mouse in particular, and other rodents and rabbits, dogs, and monkeys have been the subjects of numerous experiments, and in contrast to Man have often been successfully transplanted in various ways in a number of haemopoietic disorders. The main areas of investigation have centred around the value of bone-marrow transplantation in irradiation damage, leukaemia, and the anaemias, but, in addition, work has been focused on other possible uses and techniques for bone-marrow transplantation.

That bone-marrow transplantation was indeed a practicable proposition was learned from irradiation experiments on mice. It was found that the death which follows a high dose of X-irradiation could be circumvented by the administration of haemopoietic cells shortly after the irradiation episode (Lorenz *et al.*, 1951). This life-saving act could be performed not only by syngeneic cells, but also by allogeneic and even xenogeneic tissue (Lorenz and Congdon, 1954). It was demonstrated that the survival was the result of the colonization of the irradiated mouse by the injected cells (Ford *et al.*, 1956). These cells retained their genotypic identity in their new hosts and so produced a chimera.

Much was gleaned from the many experiments in this field, but above all there emerged one disturbing consequence. Whereas when the donor cells were syngeneic in origin, recovery was uneventful and permanent, with allogeneic cells, though there could be long-lasting success, recovery was often marred by the appearance of an illness called variously secondary disease, homologous disease, or runt disease. In the mouse the symptoms included dermatitis, a stunted and weedy appearance, and diarrhoea, with splenomegaly and aplastic lymph nodes. The illness was often grave and could be fatal. It was established that the runting syndrome was caused by a graft versus host reaction (Billingham and Brent, 1959), and the response was due to the fact that in the life-preserving inoculum some immunologically competent components attempted (sometimes successfully) to act against and reject the host.

Disregarding runting, one obvious application for bone-marrow transplantation seemed to be in leukaemia, with irradiation to kill the leukaemic cells followed by replacement of the depleted myeloid system with normal haemopoietic tissue. Initial experiments in a murine leukaemia were hopeful (Barnes *et al.*, 1956), but subsequent experiments were not successful (Barnes and Loutit, 1957, and, for example, Maddock and Djerassi, 1958; Mathé, Amiel, and Bernard, 1960). The main obstacle again seemed to be secondary disease. There is no doubt that the injected normal haemopoietic cells implant and function, but then the graft versus host action begins. Initially, this may be providential, as it reinforces the effects of the irradiation, aiding in the final eradication of the leukaemic cells. But later, as the graft versus host reaction progresses, it causes the death of the transplanted individual. Very often death from secondary disease anticipates that which would have resulted from the untreated leukaemia (Mathé, 1961).

A more promising bone-marrow transplantation in animals is in myeloid insufficiency, and this, too, has some relevance to human disease. There are many known examples of this in the mouse. One is the genetically determined severe macrocytic anaemia found in mice of the W-series. In 1956, Russell, Smith, and Lawson irradiated these mice and administered syngeneic normal haemopoietic tissue, and subsequently obtained a normal blood picture. However, these workers were not able to transplant allogeneic tissue in their mice as they could not find a level of irradiation that would allow the acceptance of the foreign tissue without itself being lethal (Bernstein, Russell, and Lawson, 1959). They found that when syngeneic tissue was used,

Received 11 March 1970.

prior irradiation was not necessary for the success of the implant (Bernstein and Russell, 1959).

In humans, of course, the ideal situation of a donor being syngeneic with the recipient is rarely encountered, and for this reason, and for many others, further work on this type of anaemia was compelling. Seller and Polani, in the course of their experiments, were successful in transplanting allogeneic haemopoietic tissue into these severely affected homozygote anaemic mice. The immunological barrier was surmounted in three different ways. First, use was made of the brief period in the perinatal life of the mouse when it is not competent to reject foreign tissue. The allogeneic cells were introduced in the first 24 hours of life (Seller and Polani, 1966). Secondly, the mice were made tolerant to the genotype of the prospective donor, by an injection of lymphoid cells at birth and then treated with allogeneic haemopoietic cells when adult (Seller, 1968a), and finally, adult anaemic mice were immunosuppressed with antilymphocytic serum (ALS) before treatment with the allogeneic cells (Seller and Polani, 1969). These last two sets of experiments were conducted effectively with the human problem in mind. Whatever the regimen for the treatment, the outcome was always the same; there was a change in the peripheral blood picture from the anaemic type to one of a haematologically normal mouse, and this was maintained for the rest of their lives. Using both haemoglobins and chromosomes as markers, it was possible to detect the donor cells in the treated mice (Seller, 1966, 1968b). The studies with chromosomes showed that in the case of these homozygote mice, the donor cells completely replaced the defective host bone-marrow cells. As would be expected from this total myeloid replacement, the granulocytes were also of the donor type (Seller, 1968c). Furthermore, the donor cells also came to constitute a large part of the dividing population of the spleen, thymus, and lymph nodes. In contrast to the bone-marrow, however, these organs retained a small core (5–20%) of host cells which were never eliminated.

The organs of the lympho-myeloid complex were colonized in a very definite sequence. After the injection of the haemopoietic cells, the spleen was the first organ to bear large numbers of dividing donor cells, and this was soon followed by the bone-marrow, then the thymus, and finally the lymph nodes (Seller, 1970b). This donor cell invasion did not extend to every tissue of the body, however, for at least one, the cornea of the eye, remained solely of host type cells in the haemopoietic chimeras (Seller, in preparation).

The heterozygote mice of the W series (genotype W^vw) are also anaemic, but are less severely affected than the homozygotes. These too were successfully transplanted and attained a normal blood picture (Seller, 1967). In this case, the bone-marrow was not completely replaced; approximately 10% of the dividing population remained of the host type.

As far as the applicability to similar human anaemias is concerned, the only model which is practically possible as yet is the one involving the use of ALS, for the period of immunological incompetence in Man ends early in fetal life. However, ALS has the disadvantage that though it is a powerful immunosuppressive and permits the establishment of the foreign cells, it itself causes an anaemia (Seller and Polani, 1969). Gengozian and Congdon (1969) have also pointed out the toxicity of ALS to haemopoietic tissue, and De Meester and Anderson (1968) feel that it probably acts directly on the stem-cells. So this may be a limiting factor in the usefulness of ALS in bone-marrow transplantation. However, ALS does have advantages. It has the ability of erasing immunological memory (Lance, 1968). Therefore, individuals unsuccessfully grafted the first time can be treated with ALS and then regrafted. This has been achieved in the W-mice (Seller, in preparation). Such possibilities must also offer hope for polytransfused humans.

The histocompatibility system in the mouse is as complex as in the human, with several loci and multiple alleles. Though the genetic relation between donor and host needs to be close, it appears that absolute identity between donor and recipient is not absolutely essential for successful bone-marrow transplantation. Russell and Bernstein (1967) have found acceptance of the bone-marrow graft in W anaemic mice when the donor differed from the host at a non H-2 locus, provided there was identity at the main H-2 locus. It is to be noted that in this situation skin grafts were rejected, albeit slowly, so the fate of skin grafts is not necessarily a reliable guide as to the potential success of a haemopoietic tissue graft.

A genetic disorder in the deer mouse, identical to human hereditary spherocytosis, has also been cured by bone-marrow transplantation (Steinmuller and Motulsky, 1967), but another murine anaemia—the Steel anaemia—has been found by McCulloch *et al.* (1965) to be resistant to such a cure. Outwardly, the Steel anaemia mimics the W anaemia, but basically the two are quite different. The W anaemia results from the gene defect acting in the haemopoietic cells themselves, delaying maturation, while the Steel anaemia is the consequence of an environment that is not sufficient to promote the

proliferation and differentiation of the erythrocyte precursor cell. The haemopoietic cells themselves are quite normal. Once released from their own defective environment they function perfectly; in fact, McCulloch *et al.* (1965) have found that they can even be used to cure the W anaemia. There are at least 11 anaemias of differing forms in the mouse. Bernstein and Webb (1965) have investigated some of these, and found that only those that are deficient in stem-cell numbers can be cured by cellular transplantation. Thus, when bone-marrow transplantation is contemplated as a form of therapy, due consideration must be paid to the inherent nature of the condition to be treated.

Another type of disease that has been investigated is found in the New Zealand Black mice. From the age of about 3 months, these animals develop a Coombs positive haemolytic anaemia, which is similar to the human autoimmune haemolytic anaemia of the warm antibody type (Holborow and Denman, 1967). Recently, Lindsey and Woodruff (1968) have succeeded in transforming some of these mice to a Coombs negative state with loss of symptoms of the disease, by transplanting bone-marrow and spleen cells from normal mice into recipients prepared by irradiation. Cells of donor origin were shown to persist in the lymphoid and myeloid systems.

Animals are of value not only in providing actual models of diseases found in humans, but also in other ways. The fact that graft versus host disease is often a limiting factor in the success of bone-marrow transplantation has already been mentioned. This disease and possible remedies have been extensively studied in animals. The exact mechanism for the disease is unknown, but direct interaction between host and donor lymphocyte populations occurs. Large numbers of donor cells are found in the lymph nodes of affected individuals, but not in the spleen. The splenomegaly that occurs as part of the disease is caused by the proliferation of the host cells and an increase in the substance of the organ itself (Nowell and Defendi, 1964). The disease can be reduced in severity by the administration of various immunosuppressive agents, for example amethopterin (Russell, 1961) or prednisone given at the same time as the haemopoietic cells, and methotrexate given at the time when the symptoms of the disease are expected (Schwartz and Beldotti, 1965). ALS has come to the fore in this respect too. In mice, treatment of the donors with ALS before they surrender their haemopoietic tissue leads to almost complete prevention of secondary disease in irradiated recipients, yet there is no decrease in the survival rate of the mice (van Bekkum *et al.*, 1967). However, as yet, there should not be too much optimism, for secondary disease is still an important reason for failure in human bone-marrow transplantation. Van Bekkum *et al.* (1969) point out that there are basic differences in the secondary disease suffered by mice and men, notably in onset and severity. Monkeys resemble man much more closely in these aspects, and so perhaps non-human primates rather than rodents should be used for finally overcoming this particular problem.

Nature's own bone-marrow transplantation experiment in the form of fetal parabiosis resulting in chimerism, first observed in fraternal cattle twins (Owen, 1945), suggested that parabiosis might be a usable technique. In mice, it is marginally successful when the histocompatibility differences are not great, for instance when the connected individuals differ in the Y-antigen (Lustgraaf, Fuson, and Eichwald, 1960) or other non H-2 loci (Martinez *et al.*, 1960). However, in allogeneic combinations, parabiotic intoxication, almost certainly another graft versus host reaction, occurs, leading to death of one partner (Cornelius, Yunis, and Martinez, 1967).

Successful bone-marrow chimerism leads to immunological tolerance to the genotype of the donor of the haemopoietic cells. The marmoset, in which fraternal twinning with placental vascular anastomoses occurs so often as to be the rule (Gengozian *et al.*, 1969) has, of course, haemopoietic tissue chimerism in the resulting adults, and skin grafts exchanged between the twin pairs are accepted permanently (Porter and Gengozian, 1969). Radiation chimeras (Main and Prehn, 1955) and transplanted anaemic mice (Seller, 1970a) also accept skin grafts from the donor of the haemopoietic tissue, while grafts from individuals unrelated both to the bone-marrow donor and the host are rejected, showing that there is specific rather than non-specific suppression. This tolerant state is usually detected by skin grafts. But if skin is accepted, why not organs such as the kidneys and heart? This idea immediately makes bone-marrow transplantation of additional value, not only in itself as a form of therapy for bone-marrow disease but also as a starting block for organ transplantation. This would be important because tolerance is the ideal form of immunological unresponsiveness so far discovered, because of its specific non-reactivity. The cattle twins resulting from parabiosis with permanent erythrocyte chimerism may eventually reject (after about 230 days) skin grafts exchanged between the twin partners (Stone *et al.*, 1965), but it appears that reciprocal kidney grafts are probably tolerated (Cragle and Stone, 1967). In an experimental situation, Grosjean

and Blumenstock (1967) have had limited success with this system. They made dogs tolerant by an injection of bone-marrow cells at birth, and then later transplanted lungs and kidneys. Two animals accepted these organ grafts for a considerable time. Further, St. Amand and Smith (1959) successfully transplanted donor type ovaries into radiation chimeras. However, overall, it seems that it is as difficult, or even more so, to achieve acceptance of an allogeneic haemopoietic tissue graft and, furthermore, to maintain it without untoward consequences, as it is to promote the survival of an allogeneic solid organ graft.

Bone-marrow transplantation in animals can thus be done in various ways and for many disorders and often is impressively successful. But in the translation of these animal models to Man success seems to be lost. Human bone-marrow transplantation has been tried in various malignancies and some of the bone-marrow aplasias, such as thrombocytopenia, agranulocytosis, and some anaemias (see Pegg, 1966). Certainly mice and men differ in many ways, but there may be fairly simple explanations for the disparity in the success rates. In Man bone-marrow transplantation is often a measure resorted to when other remedies have failed, so the recipient is probably a very severe and resistant case, in a poor condition as the disease is well advanced, and he may well have antibodies against a whole spectrum of transplantation antigens since he has probably been polytransfused. In addition, liberties can be taken with animals that cannot be taken with Man. Mice can be stressed by the technique to the utmost, they can be given more haemopoietic cells and immunosuppressed harder, because there are no repercussions (except in the conscience of the experimenter) if a few mice are lost in the course of an experiment.

Bone-marrow transplantation in Man has been tried increasingly over the past few years, especially by Mathé and his colleagues. Their clinical trials are always preceded by preliminary experiments in the mouse. However, they (1969) admit no real improvement in the fortunes of human bone-marrow transplantation over the past five years. Because of this situation, there is no doubt that research will continue and animal models will play an important role in this field. The least they do is to show that what Man may dream of doing to improve his own race is actually possible, even if at present this is restricted to treatment in a more lowly species. While in common with the whole field of organ transplantation problems of rejection remain paramount, perhaps hope for the future may lie in using immunological enhancement in bone-marrow transplantation. This involves the passive administration to the recipient of antibodies directed against the graft antigens, and it has been successfully used in rats to allow kidney transplantation (French and Batchelor, 1969), and in humans to prevent maternal sensitization to fetal rhesus antigens (Clarke, 1967).

MARY J. SELLER,
Paediatric Research Unit, Guy's Hospital Medical School, London S.E.1

REFERENCES

Barnes, D. W. H., Corp, M. J., Loutit, J. F., and Neal, F. E. (1956). Treatment of murine leukaemia with *x*-rays and homologous bone marrow. *British Medical Journal*, **2**, 626–627.

——, and Loutit, J. F. (1957). Treatment of murine leukaemia with *x*-rays and homologous bone marrow: II. *British Journal of Haematology*, **3**, 241–252.

Bernstein, S. E., and Russell, E. S. (1959). Implantation of normal blood-forming tissue in genetically anemic mice, without X-irradiation of the host. *Proceedings of the Society for Experimental Biology and Medicine*, **101**, 769–773.

——, ——, and Lawson, F. A. (1959). Limitations of whole-body irradiation for inducing acceptance of homografts in cases involving a genetic defect. *Transplantation Bulletin*, **6**, 106–108.

——, and Webb, C. (1965). Stem cell numbers in normal and anaemic mice. *36th Annual Report of the Jackson Laboratory*, p. 44.

Billingham, R. E., and Brent, L. (1959). Quantitative studies on tissue transplantation immunity. IV. Induction of tolerance in newborn mice and studies on the phenomenon of runt disease. *Philosophical Transactions of the Royal Society of London. Series B.*, **242**, 439–477.

Clarke, C. A. (1967). Prevention of Rh-haemolytic disease. *British Medical Journal*, **4**, 7–12.

Cornelius, E. A., Yunis, E. J., and Martinez, C. (1967). Parabiosis intoxication: clinical, hematologic and serologic features. *Transplantation*, **5**, 112–134.

Cragle, R. G., and Stone, W. H. (1967). Preliminary results of kidney grafts between cattle chimeric twins. *Transplantation*, **5**, 328–330.

De Meester, T. R., and Anderson, N. D. (1968). The potential bone marrow toxicity of antilymphocyte serum in mice. *Journal of Surgical Research*, **8**, 192–195.

Ford, C. E., Hamerton, J. L., Barnes, D. W. H., and Loutit, J. F. (1956). Cytological identification of radiation-chimaeras. *Nature (London)*, **177**, 452–454.

French, M. E., and Batchelor, J. R. (1969). Immunological enhancement of rat kidney grafts. *Lancet*, **2**, 1103–1106.

Gengozian, N., Batson, J. S., Greene, C. T., and Gosslee, D. G. (1969). Haemopoietic chimaerism in imported and laboratory bred marmosets. *Transplantation*, **8**, 633–652.

——, and Congdon, C. C. (1969). Effect of syngeneic antilymphocytic serum on rat–mouse radiation chimaeras. *Transplantation Proceedings*, **1**, 34–38.

Grosjean, O., and Blumenstock, D. (1967). Transplantations pulmonaires et rénales chez le chien traité par injection néonatale unique ou ultérieurement répétée de moelle osseuse. *Comptes Rendus des Séances de la Société de Biologie et de ses Filiales*, **161**, 733–737.

Holborow, E. J., and Denman, A. M. (1967). Autoimmune disease in inbred mice. *Clinical and Experimental Immunology*, **2**, 761–767.

Lance, E. (1968). Erasure of immunological memory with antilymphocyte serum. *Nature (London)*, **217**, 557–558.

Lindsey, E. S., and Woodruff, M. F. A. (1968). Infusion of marrow and spleen cells in irradiated NZB/Bl mice: Amelioration of autoimmune disease. *British Journal of Haematology*, **14**, 255–260.

Lorenz, E., and Congdon, C. C. (1954). Modification of lethal irradiation injury in mice by injection of homologous and heterologous bone. *Journal of the National Cancer Institute*, **14**, 955–961.

——, Uphoff, D., Reid, T. R., and Shelton, E. (1951). Modifications of irradiation injury in mice and guinea pigs by bone marrow injections. *Journal of the National Cancer Institute*, **12**, 197–201.

Lustgraaf, E. C., Fuson, R. B., and Eichwald, E. J. (1960). Sex tolerance and split tolerance. *Transplantation Bulletin*, **26**, 145–150.

McCulloch, E. A., Siminovitch, L., Till, J. E., Russell, E. S., and Bernstein, S. E. (1965). The cellular basis of the genetically determined hemopoietic defect in anemic mice of genotype Sl/Sl. *Blood*, **26**, 399–410.

Maddock, C. L., and Djerassi, I. (1958). Effects of total-body x-radiation and protection with isologous bone marrow on the growths of transplantable mouse tumors. *Proceedings of the American Association for Cancer Research*, **2**, 323–324.

Main, J. M., and Prehn, R. T. (1955). Successful skin homografts after the administration of high dosage x-radiation and homologous bone marrow. *Journal of the National Cancer Institute*, **15**, 1023–1029.

Martinez, C., Shapiro, F., Kelman, H., Onstad, T., and Good, R. A. (1960). Tolerance of F_1 hybrid skin grafts in the parent strain induced by parabiosis. *Proceedings of the Society for Experimental Biology and Medicine*, **103**, 266–269.

Mathé, G. (1961). Secondary syndrome a stumbling block in the treatment of leukaemia by whole body irradiation and transfusion of allogeneic haemopoietic cells. In *Diagnosis and Treatment of Acute Radiation Injury*, p. 191. World Health Organization, Geneva.

——, Amiel, J. L., and Bernard, J. (1960). Traitement de souris AkR à l'âge de six mois par irradiation totale suivie de transfusion de cellules hématopoïétiques allogéniques. Incidences respectives de la leucémie et du syndrome secondaire. *Bulletin de l'Association Française pour l'Étude du Cancer*, **47**, 331–340.

——, ——, Schwartzenberg, L., Schneider, M., Cattan, A., Schlumberger, Y. R., Nouza, K., and Hrask, Y. (1969). Bone marrow transplantation in man. *Transplantation Proceedings*, **1**, 16–24.

Nowell, P. C., and Defendi, V. (1964). Distribution of proliferating donor cells in runt disease in rats. *Transplantation*, **2**, 375–382.

Owen, R. D. (1945). Immunogenetic consequences of vascular anastomoses between bovine twins. *Science*, **102**, 400–401.

Pegg, D. E. (1966). *Bone Marrow Transplantation*. Lloyd-Luke, London.

Porter, R. P., and Gengozian, N. (1969). Immunological tolerance and rejection of skin allografts in the marmoset. *Transplantation*, **8**, 653–665.

Russell, E. S., and Bernstein, S. E. (1967). Influence of non-H-2 genetic factors on success in implantation therapy of *W* series hereditary anemias in the mouse. *Transplantation*, **5**, 142–153.

——, Smith, L. J., and Lawson, F. A. (1956). Implantation of normal blood-forming tissue in radiated genetically anemic hosts. *Science*, **124**, 1076–1077.

Russell, P. S. (1961). Means of modifying runt disease in mice. In *Mechanisms of Immunological Tolerance*, p. 55. Ed. by M. Hasek, A. Lengerova, and M. Vojtiskova. Academic Press, New York and London.

St. Amand, W., and Smith, L. H. (1959). Interstrain ovarian transplantation in lethally irradiated mice after bone marrow therapy. *Nature (London)*, **184**, 1503.

Schwartz, R. S., and Beldotti, L. (1965). The treatment of chronic murine homologous disease: a comparative study of four 'immunosuppressive' agents. *Transplantation*, **3**, 79–97.

Seller, M. J. (1966). Donor haemoglobin in anaemic mice of the W-series transplanted with haematopoietic tissue from an unrelated donor. *Nature (London)*, **212**, 81–82.

—— (1967). Experimental red cell chimerism in the heterozygote (W^vw) of the W-series mutants in the house mouse. *Journal of Medical Genetics*, **4**, 33–35.

—— (1968a). The transplantation of allogeneic haemopoietic tissue in adult anaemic mice of the W-series previously made immunologically tolerant. *Transplantation*, **6**, 856–860.

—— (1968b). Transplantation of anaemic mice of the W-series with haemopoietic tissue bearing marker chromosomes. *Nature (London)*, **220**, 300–301.

—— (1968c). An attempt at colonisation of W^vW^v anemic mice by rat hemopoietic tissue. *Experientia*, **24**, 954–955.

—— (1970a). The establishment of tolerance in adult anaemic mice of the W-series treated with antilymphocytic serum and allogeneic haemopoietic cells. *Clinical and Experimental Immunology*, **6**, 639–643.

—— (1970b). The speed and order of colonisation of some organs of anaemic mice of the W-series transplanted with allogeneic haemopoietic tissue. *Transplantation*, **9**, 303–309.

——, and Polani, P. E. (1966). Experimental chimerism in a genetic defect in the house mouse *Mus musculus*. *Nature (London)*, **212**, 80–81.

——, and —— (1969). Transplantation of allogeneic haemopoietic tissue in adult anaemic mice of the W-series using antilymphocytic serum. *Lancet*, **1**, 18–21.

Steinmuller, D., and Motulsky, A. G. (1967). Treatment of hereditary spherocytosis in Peromyscus by irradiation and allogeneic bone marrow transplantation. *Blood*, **29**, 320–330.

Stone, W. H., Cragle, R. G., Swanson, E. W., and Brown, D. G. (1965). Skin grafts: delayed rejection between pairs of cattle twins showing erythrocyte chimerism. *Science*, **148**, 1335–1336.

van Bekkum, D. W., Balner, H., Dickie, K. A., and van Putten, L. M. (1969). Experimental aspects of bone marrow transplantation in primates. *Transplantation Proceedings*, **1**, 25–30.

——, Ledney, G. D., Balner, H., van Putten, L. M., and de Vries, M. J. (1967). Suppression of secondary disease following foreign bone marrow grafting with antilymphocyte serum. In *Antilymphocytic Serum*, p. 97. Ed. by G. E. W. Wolstenholme and M. O'Connor. Churchill, London.