The Application of Bone Marrow Transplantation to the Treatment of Genetic Diseases

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Genetic diseases can be treated by transplantation of either normal allogeneic bone marrow or, potentially, autologous bone marrow into which the normal gene has been inserted in vitro (gene therapy). Histocompatible allogeneic bone marrow transplantation is used for the treatment of genetic diseases whose clinical expression is restricted to lymphoid or hematopoietic cells. The therapeutic role of bone marrow transplantation in the treatment of generalized genetic diseases, especially those affecting the central nervous system, is under investigation. The response of a generalized genetic disease to allogeneic bone marrow transplantation may be predicted by experiments in vitro. Gene therapy can be used only when the gene responsible for the disease has been characterized. Success of gene therapy for a specific genetic disease may be predicted by its clinical response to allogeneic bone marrow transplantation.

HISTOCOMPATIBLE BONE MARROW TRANSPLANTATION IS the treatment of choice for patients with a variety of hematological, immunological, and oncological diseases that are not of genetic origin (*1*). Since the first successful allogeneic bone marrow transplant was performed in a child with severe combined immune deficiency (SCID) in 1968, more than 5000 bone marrow transplants have been performed (*2*). Allogeneic or autologous bone marrow transplants are now used in an attempt to cure or stabilize patients with genetic diseases. The technology of allogeneic transplantation for genetic diseases is similar to transplantation for other diseases. Before undergoing transplantation, the recipients are prepared by subjecting them to chemotherapy with or without total body irradiation (TBI). They then receive an intravenous infusion of histocompatible bone marrow, whereupon hematological and lymphoid engraftment are achieved. However, the recipients are immunodeficient and may develop graft versus host disease (GVHD) although most of them can be discharged from the hospital 30 to 60 days after transplantation.

The likelihood of an allogeneic bone marrow transplant curing or stabilizing a particular genetic disease depends on (i) the tissue-specific expression of the normal gene product, (ii) the clinical symptomatology of the disease, and (iii) the cellular transport of the normal gene product. The response of a patient with a particular genetic disease to allogeneic bone marrow transplantation may predict whether the disease will respond to the transplantation of autologous bone marrow into which the normal gene has been inserted in vitro (gene therapy). Table 1 gives a classification of genetic diseases based on their clinical symptoms and their response to allogeneic bone marrow transplantation.

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Restricted Genetic Diseases

The first genetic disease to be completely corrected by allogeneic bone marrow transplantation was SCID. Infants with SCID lack functional B and T lymphocyte immunity. Thus, like goiter, SCID is a clinical phenotype due to a spectrum of primary defects. In most SCID patients, expression of the primary defect is restricted to lymphoid cells (class I disease) although one form of the disease, adenosine deaminase (ADA) deficiency, is characterized by a generalized absence of the enzyme (class IV disease) (*3*). The transplantation of histocompatible normal bone marrow containing both lymphoid and hematopoietic stem cells into SCID patients results in establishment of the donor lymphoid cells in 90% of patients as determined by chromosomal analysis of T lymphocytes. The engraftment of hematopoietic stem cells (granulocytes, erythrocytes, and platelets) is not sustained in SCID patients, who do not receive chemotherapy prior to the operation or who do not develop acute GVHD (*4*). This suggests that engraftment of the donor lymphoid stem cells is selective or that the engrafted stem cells differentiate only along lymphoid lines. If the hematopoietic stem cells of the recipient are ablated by chemotherapy (busulfan) or TBI prior to transplantation or by GVHD after transplantation, donor lymphoid and hematopoietic engraftment is achieved. Thus, the infusion of histocompatible bone marrow results in the engraftment and differentiation of only those stem cells for which there is "physiological space."

In the late 1960's and early 1970's, bone marrow transplants were attempted in patients with genetic defects of the hematopoietic stem cell (platelets in the Wiskott-Aldrich syndrome and granulocytes in chronic granulomatous disease) (*5*). Hematopoietic stem cell engraftment was not sustained, however, primarily because of the continued presence of the abnormal recipient hematopoietic stem cells. In 1977, the first complete correction of a genetic defect involving the hematopoietic stem cell was achieved. A patient with the Wiskott-Aldrich syndrome, which includes abnormalities in both T lymphocytes (lymphoid stem cell defect) and platelets (hematopoietic stem cell defect) (class II disease), was prepared for transplantation with cyclophosphamide as an immunosuppressive

agent and high doses of arabinosylcytosine as an anti-hematopoietic stem cell agent (*6*). The infusion of histocompatible normal female bone marrow resulted in a temporary T lymphocyte graft, but no evidence of hematopoietic engraftment. After the loss of the T lymphocyte graft, the patient was prepared for a second transplant with increased immunosuppression [cyclophosphamide, rabbit antiserum to human thymocytes (ATS), and procarbazine] and 750 R of TBI, which in leukemic patients had been shown to ablate normal hematopoiesis. The subsequent transplantation with bone marrow from the same donor resulted in complete donor lymphoid and hematopoietic engraftment, with all T and B lymphocytes and all hematopoietic elements (erythrocytes, granulocytes, and platelets) being of donor origin. More than 40 patients with the Wiskott-Aldrich syndrome have received transplants worldwide during the last 10 years with more than a 90% cure rate (*7*).

The initial use of TBI as an anti-hematopoietic stem cell agent has been superseded by the use of busulfan which, in adequate doses, is as effective as TBI in eradicating the abnormal hematopoietic stem cells but has less clinical toxicity, particularly with regard to its causing idiopathic interstitial pneumonitis (*8*). Thus, preparation with a combination of drugs that have anti-lymphoid and anti-hematopoietic stem cell activities creates adequate "physiological space" for the engraftment of the normal donor lymphoid and hematopoietic stem cells. At present, no single agent or drug has been shown to have both adequate immunosuppressive and anti-hematopoietic stem cell activity to ensure engraftment. Therefore, a combination of agents or drugs must be used. Either of the combinations, ATS and TBI or cyclophosphamide and busulfan, ensures the eradication of the abnormal recipient lymphoid and hematopoietic stem cells.

The success of transplantation for class I or class II diseases is aided by the primary immunodeficiency associated with the defective lymphoid stem cell. Conversely, the success of transplantation for genetic diseases restricted to cells of hematopoietic origin (class III diseases) is complicated by the presence of normal lymphoid immunity. In patients with aplastic anemia, transfusions prior to transplantation increase the rate of marrow graft rejection unless the immunosuppressant regimes prior to transplantation are intensified (*9*). Transplantation for genetic diseases restricted to the hematopoietic system, in which patients receive blood transfusions prior to transplantation (erythrocytes in thalassemia, granulocytes in infantile agranulocytosis, and platelets in congenital amegakaryocytosis), have the additional risk of marrow graft rejection. The first genetic diseases involving cells of only hematopoietic origin to be treated successfully by transplantation were disorders of myeloid differentiation and function. Infantile agranulocytosis (Kostmann's syndrome), granulocyte actin deficiency, and chronic granulomatous disease were all corrected after the ablation of the abnormal recipient hematopoietic stem cells by TBI or busulfan and adequate immunosuppression to eradicate the normal lymphoid stem cells (*10*).

Tissue macrophages (osteoclasts, Kupffer's cells, alveolar macrophages, Langerhans' cells, and possibly microglial cells) are of hematopoietic stem cell origin. After ablation of hematopoietic stem cells in the recipient and engraftment of donor hematopoietic cells, repopulation with tissue macrophages of donor origin occurs. The turnover of the tissue macrophages is slower than that of circulating monocytes and macrophages. Although all circulating granulocytes and monocytes are of donor origin by 2 to 3 weeks after transplantation, the tissue macrophages continue to be of recipient origin for 3 to 6 months (*11*). Thus osteopetrosis, in which there is abnormal osteoclast activity, can be totally corrected by allogeneic bone marrow transplantation if the transplant is performed before irreversible neurological damage occurs (*12*). The improvement of patients with osteopetrosis, as measured by an increase in calcium excretion or the normalization of the marrow cavity, starts 3 to 4 months after transplantation.

In addition to defects of myeloid function, primary defects of erythroid differentiation and hemoglobin structure and regulation can be corrected by allogeneic bone marrow transplantation. Transplantation has been used to treat more patients with thalassemia than with any other erythroid-restricted genetic disease (*13*). This treatment has also been used successfully for patients with sickle cell anemia and pure red cell aplasia (*14*). The use of transplantation for older patients with thalassemia is complicated by the development of cardiac toxicity secondary to iron overload and graft rejection due to T lymphocyte sensitization, both of which are due to the patients' repeated blood transfusions. Bone marrow transplantation for older patients with thalassemia initially had a success rate of 15% (*13*). Recent transplants with younger patients (6 months to 10 years old), who have received fewer blood transfusions, have an actuarial disease-free survival rate of 73% (*15*). Thus, the early use of transplantation for patients with genetic diseases, before there is clinical deterioration due to the primary disease and its concomitant therapy, leads to improved clinical results.

Because of the clinical heterogeneity of patients with genetic defects, it is difficult to make decisions on the applicability of bone marrow transplantation. Among the factors considered for each individual patient are (i) the success of bone marrow transplantation in curing that disease in previous patients, (ii) the patient's present clinical condition, and (iii) the expected results with alternative therapy, for example, hypertransfusion and iron chelation therapy for thalassemia.

Generalized Genetic Diseases

Some genetic diseases are characterized by a generalized absence of a specific enzyme (ADA deficiency in SCID and glucose cerebrosidase in Gaucher's disease), but the clinical symptomatology is restricted primarily to cells of lympho-hematopoietic origin (T lymphocytes in ADA deficiency and tissue macrophages in Gaucher's disease) (class IV diseases). The transplantation of normal lymphoid stem cells into ADA-deficient SCID patients results in normal T lymphocyte immunocompetence even though there is no correction of the ADA deficiency in the erythrocytes, granulocytes, or other nonlymphoid tissues (*16*). Thus, the presence of normal ADA concentrations in lymphoid tissues permits normal T lymphocyte differentiation even though elevated levels of adenosine metabolites are present in other tissues. Much of the clinical symptomatology of Gaucher's disease, types 1 and 3, is due to tissue macrophages filled with undegraded glucocerebroside. The ablation of the abnormal hematopoietic stem cells by the administration of busulfan in conjunction with immunosuppressive agents has permitted hematopoietic engraftment from healthy donors (*11*). In one patient so treated, normalization of peripheral blood leukocyte glucocerebrosidase activity occurred within 1 month of transplantation. However, the disappearance of Gaucher's cells from the bone marrow did not start until 5 months after transplantation, demonstrating that the turnover of tissue macrophages was markedly slower than that of the circulating monocytes. The results with bone marrow transplantation in Gaucher's disease are superior to those seen with the transplantation of spleen or kidney, where graft maintenance is difficult and normalization of circulating enzyme levels is not achieved (*17*). The infusion of exogenous glucocerebrosidase decreases glucocerebroside accumulations; however, the decreases are less than those occurring after bone marrow transplantation (*18*). Thus, allogeneic bone marrow transplantation is the most effective therapy available for severe Gaucher's disease.

Fanconi anemia is a generalized genetic disease characterized by increased DNA fragility, but the primary defect is unknown. Affected individuals develop pancytopenia due to hypoproduction of all hematopoietic elements by the abnormal hematopoietic stem cells. The ablation of the affected stem cells followed by the transplantation of normal bone marrow results in normal hematopoiesis. However, the increased propensity of the patient's DNA to damage by chemotherapy and irradiation necessitates a decrease in the intensity of the preparation of the patients (*19*).

The role of allogeneic bone marrow transplantation in the correction of genetic diseases with generalized expression and clinical symptomatology in non–lympho-hematopoietic tissues (class V and VI diseases) is complicated. Bone marrow transplantation has a potential role in the treatment of generalized diseases if normal lympho-hematopoietic cells produce the missing or defective enzyme (class V diseases). If the enzyme is not expressed in normal bone marrow–derived cells, transplantation has no therapeutic role (class VI diseases). The establishment of donor lymphoid and hematopoietic engraftment in a patient with phenylketonuria would have no therapeutic effect since the bone marrow–derived cells would not contain phenylalanine hydroxylase.

The basic assumption behind the therapeutic role for allogeneic bone marrow transplantation in the treatment of enzyme deficiency diseases is that bone marrow–derived cells from normal or heterozygote donors are an ongoing source of the normal enzyme and that the circulating donor enzyme is capable of gaining access to the sites of substrate accumulation in adequate concentrations to produce clinically significant substrate degradation. Thus, the likelihood of circulating enzyme producing clinical benefit can be predicted by (i) previous clinical results with normal plasma or enzyme infusions, (ii) the relative amounts of enzyme in lympho-hematopoietic cells compared to the affected tissue (liver, muscle, heart), (iii) the exposure of the affected organ to circulating enzyme (the blood-brain barrier must be considered), (iv) whether any central nervous system (CNS) cells are of lympho-hematopoietic origin (for example, microglial cells), and (v) the presence of transport mechanisms for the cytoplasmic accumulation of the circulating enzyme.

Hurler's syndrome (mucopolysaccharidosis type 1-H), a disorder of mucopolysaccharide metabolism, was an early candidate for treatment by allogeneic bone marrow transplantation since prior clinical results with plasma infusions and fibroblast grafts had demonstrated that exogenous α-L-iduronidase was able to increase the urinary excretion of mucopolysaccharide (*20*). Furthermore, fibroblast coculture experiments had demonstrated that the normal enzyme was capable of gaining access to the intracellular accumulations of mucopolysaccharide, indicating the presence of a transport mechanism for α-L-iduronidase (*21*). It is not surprising, therefore, that the engraftment of normal bone marrow cells resulted in the increased urinary excretion of mucopolysaccharide and the disappearance of extra-CNS symptomatology (hepatosplenomegaly and corneal clouding) (*22*). However, the efficacy of the circulating enzyme in reaching the CNS accumulations of mucopolysaccharide is still uncertain. Longitudinal follow-up of the patients with Hurler's syndrome who received transplants early in their clinical course is needed to determine if hydrocephalus can be prevented and normal intellectual function maintained. Such studies are being conducted. Circulating enzyme may gain access to the CNS poorly because of the blood-brain barrier. That the blood-brain barrier may be circumvented is suggested by the fact that microglial cells, like osteoclasts and Kupffer's cells, may be of bone marrow origin. Nine months after allogeneic bone marrow transplantation in mice, Ia (I-region associated) positive CNS cells (microglial cells) of donor origin are detected, suggesting that the turnover of CNS bone marrow–derived cells is slow (*23*). If microglial cells are of bone marrow origin, transplanted microglial cells could be a continuing source for the local production and release of the normal enzyme.

Recent evidence has suggested that there may be a CNS hematopoietic stem cell that differs from the bone marrow hematopoietic cell (*24*). The mice, in which successful repopulation of the CNS with donor-derived bone marrow cells occurred, were prepared for transplantation with TBI in lethal amounts. Most patients receiving bone marrow transplants for genetic diseases are now prepared with busulfan (*8*). It is possible that busulfan may not reach the brain in adequate concentrations to eliminate the CNS hematopoietic stem

Table 1. Classification of some of the genetic diseases that may be treated by bone marrow transplantation (BMT).

Class I	Class II	Class III	Class IV	Class V	Class VI
			Expression		
Expression of genetic defect restricted to lymphoid cells	Expression of genetic defect restricted to lymphoid and hematopoietic cells	Expression of genetic defect restricted to hematopoietic cells	Generalized genetic defect; clinical symptomatology restricted to lympho-hematopoietic cells	Generalized genetic defect; generalized clinical symptomatology with or without CNS involvement	Lympho-hematopoietic cells do not express the normal gene product
			Treatment		
Correctable by BMT	Correctable by BMT	Correctable by BMT	Correctable by BMT	May be correctable by BMT	Not correctable by BMT
			Disease		
Severe combined immune deficiency (non-ADA deficient)*	Wiskott-Aldrich syndrome*	Thalassemia*	Gaucher's disease*†	Adrenoleukodystrophy*	Cystic fibrosis
	Chédiak-Higashi syndrome*	Granulocyte actin deficiency*	Adenosine deaminase deficiency*†	Metachromatic leukodystrophy*	Hemophilia
gpL-115 deficiency*		Chronic granulocyte disease*	Nucleotide phosphorylase deficiency*†	Krabbe's disease	Phenylketonuria
X-linked agammaglobulinemia		Infantile agranulocytosis*	Fanconi anemia*	Mucopolysaccharidosis*†	
		Sickle cell disease*		Lesch-Nyhan syndrome*†	
		Osteopetrosis*			

*Diseases for which allogeneic bone marrow transplantation has been attempted. †Candidate diseases for gene therapy.

cell if such a separate CNS stem cell population does exist. The presence of a separate CNS stem cell population might require local irradiation to the brain in addition to systemic busulfan in order to permit the repopulation of the CNS with donor-derived cells. After the treatment of patients with Hurler's syndrome by transplantation, other patients with mucopolysaccharidoses, including those with Hunter's, Sanfilippo B, and Maroteaux-Lamy syndromes, have been treated by transplantation, and non-CNS symptomatology has been corrected (*25*). Since patients with Maroteaux-Lamy syndrome have minimal CNS involvement, they are particularly good candidates for treatment with allogeneic bone marrow.

Because it proved possible to correct in vitro the cerebroside sulfate metabolism of fibroblasts from patients with metachromatic leukodystrophy (LMD) by exposing them to exogenous arylsulfatase A, it was suggested that LMD might be corrected by allogeneic bone marrow transplantation (*26*). Successful hematopoietic engraftment of such an LMD patient was then achieved and resulted in the patient's developmental quotient becoming stabilized and his electrophysiological abnormalities corrected 6 months after transplantation (*27*). The lag in the patient's clinical stabilization and in the appearance of mononuclear cells of donor origin in the patient's cerebrospinal fluid indicates that the repopulation of the CNS with cells of donor origin was delayed. Allogeneic bone marrow transplantation for CNS storage diseases needs to be performed before significant clinical deterioration occurs. Unlike diseases such as thalassemia or infantile agranulocytosis, clinical improvement or stabilization of CNS storage diseases does not occur immediately after engraftment.

Animal experiments with the twitcher mouse, a model for galactocerebrosidase deficiency (Krabbe's disease), have indicated that normal enzyme accumulates slowly in affected neurons after their transplantation into normal mice (*28*). This slow rate of enzyme accumulation, together with the slow turnover of bone marrow–derived cells within the CNS, suggests that it may be difficult for clinically significant concentrations of the normal enzyme to accumulate in the neurons and other CNS cells before irreversible CNS disease occurs.

Another method to evaluate the potential role of allogeneic bone marrow transplantation for the treatment of enzyme deficiency diseases is to examine the clinical status of heterozygous individuals or patients with "partial defects." Since bone marrow–derived cells represent only 10% of the total body cellular mass, the engraftment of enzymatically normal bone marrow will result in a total body enzyme content that is only 10% of normal, assuming that the enzyme is equally expressed in all cells. Therefore, if heterozygous individuals with enzyme levels that are 10 to 30% of normal display significant clinical symptomatology, complete engraftment of donor cells, with a resultant enzyme content that is 10% of normal, will be unlikely to produce clinical improvement. Conversely, if patients with partial defects and a low level of enzyme activity (1 to 5% of normal) are clinically normal, allogeneic bone marrow transplantation may have a therapeutic role.

Patients with partial defects in hypoxanthine–guanine phosphoribosyltransferase (HGPRT) have the same choreoathetoid movements and defects in uric acid metabolism as patients with a complete absence of HGPRT (Lesch-Nyhan syndrome) but do not undergo the self-mutilation that characterizes complete HGPRT deficiency (*29*). The establishment of normal bone marrow function might, therefore, eliminate the self-mutilating behavior even though the other components of the disease would persist. Donor bone marrow engraftment with the presence of normal peripheral blood leukocyte HGPRT levels has not produced clinical improvement in a patient with HGPRT deficiency (*30*). Possible reasons for the lack of clinical improvement are that (i) the leukocyte HGPRT did not reach the CNS, (ii) irreversible brain damage had occurred prior to transplantation even though anatomically detectable damage was not apparent, or (iii) a longer period is required for adequate levels of HGPRT to be achieved. Transplantation in younger patients with HGPRT deficiency may have to be undertaken before a definitive statement about the role of allogeneic bone marrow transplantation in the treatment of HGPRT deficiency can be made.

Patients with Pompe's disease (α-1,4-glucosidase deficiency) have been treated with exogenous enzyme with an increase in liver enzyme activity and a decrease in liver glycogen content (*31*). Electron microscopic examination of liver biopsies after enzyme therapy demonstrated that, although lysosomal accumulation of glycogen had been reduced, cytoplasmic and membrane glycogen accumulations were unchanged. These results in vivo, in conjunction with studies of affected fibroblasts in vitro, have indicated that the glycogen degradation was due to exogenous enzyme that had undergone pinocytosis and that no specific transport mechanism for exogenous enzyme exists. The lack of cytoplasmic enzyme activity or glycogen degradation suggested that allogeneic bone marrow transplantation might produce little clinical benefit. The presence of normal peripheral blood leukocyte glucosidase activity after allogeneic bone marrow transplantation has resulted in no increase in muscle or liver enzyme activity nor any decrease in muscle or liver glycogen content (*32*). Thus, the response of a particular genetic disease to allogeneic bone marrow transplantation may be predicted by experiments in vitro.

Limitations

Even if a specific genetic disease is treatable by allogeneic bone marrow transplantation, such treatment is limited by (i) the possibility of bone marrow graft rejection, (ii) the possibility of post-transplant GVHD, and (iii) donor availability. Diseases in which patients are transfused prior to transplantation are at an increased risk of marrow graft rejection. All recipients may develop GVHD due to the attack by immunocompetent donor T lymphocytes against recipient-specific non-HLA (histocompatibility) antigens. The severity of GVHD increases with increasing recipient age, suggesting that the best clinical results of transplantation will occur in young patients (*33*). Patients who receive transplants for genetic diseases usually receive bone marrow from histocompatible siblings, who share the same HLA-A, -B, -C, and -Dr antigens. The probability that any two siblings are histocompatible is 1 in 4 and, therefore, only 25 to 30% of patients with treatable diseases have a histocompatible donor. The transplantation of T lymphocyte–depleted haplo-identical parental bone marrow has had little success in patients with genetic diseases other than SCID because of the presence of normal recipient lymphoid immunity that cannot be adequately suppressed (*34*). These limitations on the use of allogeneic bone marrow transplantation have increased interest in the infusion of autologous bone marrow after the insertion of the normal gene in vitro (gene therapy).

Gene Therapy

The use of transfected autologous bone marrow has several advantages compared to allogeneic bone marrow for the correction of genetic diseases. After autologous bone marrow transplantation there is no possibility of an immunologically mediated rejection unless the inserted gene modifies the surface antigens of the stem cells. GVHD will not occur, and all patients have a potential donor, themselves. In spite of these advantages, gene therapy will probably

not produce the same degree of correction as the engraftment of normal allogeneic bone marrow since at present the normal gene cannot be inserted into all stem cells and the expression of the gene product in the transfected cells is less than that in normal cells (*35*). It will not be possible to use gene therapy to treat class VI diseases that cannot be corrected by allogeneic bone marrow transplantation unless techniques are developed that will permit the expression of genes (for example, phenylalanine hydrolase) that are not usually expressed in lympho-hematopoietic cells (*36*).

The prospects for gene therapy with special emphasis on the technology of gene insertion were recently reviewed (*37*). The results of allogeneic bone marrow transplantation provide information about the efficacy and problems associated with gene therapy and can thus be used as a "screen" for genetic diseases that may benefit from gene therapy. Three diseases frequently considered as candidates for gene therapy are ADA deficiency, nucleotide phosphorylase (NP) deficiency, and HGPRT deficiency. ADA and NP deficiencies clinically involve only lymphoid cells even though they are generalized enzymatic deficiencies; both have been corrected by allogeneic bone marrow transplantation. HGPRT deficiency is a generalized enzymatic deficiency with generalized symptomatology including CNS involvement; although one patient who received a transplant for HGPRT deficiency achieved lymphoid and hematopoietic engraftment, no clinical improvement has yet been detected.

Some investigators have thought that chemotherapy prior to transplantation may not be required for the transplantation of autologous bone marrow since the stem cells with the inserted normal gene may have a selective advantage over the abnormal stem cells. In diseases such as ADA and HGPRT deficiencies, the normal lymphoid or hematopoietic cells may have a selective advantage, especially if selective pressure (methotrexate in HGPRT deficiency) is applied. In other diseases (thalassemia and Gaucher's disease) no selective advantage would exist, and the ablation of the abnormal stem cells would be necessary. Stem cells undergo differentiation and self-renewal after engraftment. If the transfected stem cells have a selective advantage in differentiation or self-renewal, they probably have no selective advantage at the stage of engraftment since, presumably, there is no "physiological space." Recipients of gene therapy transplants will probably have to receive some form of pretransplantation chemotherapy to provide "space" for the engraftment of the transfected stem cells. However, partial rather than total elimination of the patient's stem cells may be possible. Patients with Gaucher's disease might need to have only their hematopoietic stem cells ablated with the preservation of their mature lymphocytes and lymphoid stem cells. The maintenance of the lymphoid cells would reduce the immunodeficiency that occurs after transplantation.

Another prerequisite when gene therapy is being considered is a clear understanding of the pathophysiology of the disease. The Wiskott-Aldrich syndrome has the highest cure rate of all genetic diseases following allogeneic bone marrow transplantation. However, the underlying primary defect in the Wiskott-Aldrich syndrome, probably an abnormality in a 115,000-dalton glycoprotein in lymphocytes and GP Ia-Ib–related glycoprotein in platelets, is poorly understood (*38*). If the normal gene has not been cloned, gene therapy is not possible. Therefore, in spite of the superior results with allogeneic transplantation, the Wiskott-Aldrich syndrome is not a candidate for gene therapy at present. The non-ADA–deficient forms of SCID, chronic granulomatous disease and osteopetrosis, present similar difficulties. Thus, identification of the basic defect in a genetic disease is a prerequisite for its potential treatment by gene therapy.

Diseases that are due to defects in structural genes (ADA deficiency, NP deficiency) and are clinically restricted to lympho-hematopoietic cells (class I, II, III, and IV diseases) are the best candidates for gene therapy since the site of the insertion of the normal gene may not be critical for gene expression. Genetic defects of genes expressed in a tissue-specific manner (thalassemia) will be more difficult to correct since the precise insertion of the normal gene may be required to ensure the normal intranuclear regulation of genetic activity. Additional problems associated with the regulation of transfected gene expression have been demonstrated in transgenic mice in which the transcription of the inserted gene is regulated by *trans* regulatory elements and the phenotypic expression of the inserted gene is less than anticipated (*39*).

The use of gene therapy for genetic diseases that clinically affect non–lympho-hematopoietic cells will have the same problems as allogeneic transplantation; that is, success will depend on whether the lympho-hematopoietic–derived enzyme can gain access to substrate accumulations. Since the total production of the normal enzyme by the transplanted cells may be less after gene therapy than after allogeneic transplantation, the results with gene therapy may be inferior except for diseases where low levels of exogenous enzyme are corrective. The potential problems associated with the blood-brain barrier and the CNS storage of substrate will still exist.

Conclusion

Genetic diseases, whose clinical expression is restricted to cells of lympho-hematopoietic origin, can be cured by the transplantation of normal histocompatible bone marrow after ablation of the abnormal lymphoid or hematopoietic stem cells, or both. The use of allogeneic bone marrow transplantation for the treatment of generalized genetic diseases is based on the assumption that the donor lympho-hematopoietic cells are an ongoing source of normal enzyme. The success of bone marrow transplantation for a specific generalized genetic disease depends in part on whether mechanisms exist for the transport of the circulating enzyme to the site of substrate accumulation. Further research into the transport of enzymes will permit a more accurate prediction of which genetic diseases will benefit from allogeneic bone marrow transplantation. For genetic diseases involving the CNS, bone marrow transplantation is complicated by the role of the blood-brain barrier in preventing circulating enzyme from entering the CNS and by whether bone marrow–derived cells are normally present in the CNS to provide the local production of enzyme. Allogeneic transplantation for genetic diseases is limited by donor availability. The use of autologous bone marrow after the insertion of a normal gene (gene therapy) in vitro circumvents the need for a histocompatible donor. However, results with gene therapy may be inferior to those achieved with allogeneic transplantation because of the lack of insertion of the normal gene into all the transplanted stem cells and because of a lower level of gene expression. It will be necessary to understand the primary defect of many genetic diseases that are now treated by allogeneic bone marrow transplantation for the full potential of gene therapy to be realized.

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40. The assistance of R. Baehner, W. Nyhan, and K. Weinberg in reviewing this article, D. G. Nathan and F. S. Rosen in many discussions, and K. Cooper in its preparation is gratefully acknowledged. This work was supported in part by NIH funds.