

I. LAY SUMMARY

Gaucher disease is an inherited disease. Patients suffer from enlarged organs, bone deterioration with multiple fractures and in some patients, progressive neurological degeneration. Most patients suffer significant disability and, many, an early death. The symptoms of Gaucher disease are a consequence of the accumulation of a fatty substance within specialized house keeping cells called macrophages(MØ). Bone marrow transplantation (BMT) results in the replacement of sick MØ by normal ones. It has resulted in significant clinical improvement for some patients. Unfortunately the therapy is not available for the majority of patients because they lack a suitable bone marrow donor. The development of macrophage targeted enzyme replacement therapy has provided treatment options, but is not a completely satisfactory solution. Although patients improve with enzyme treatment, the therapy is not permanent and is very expensive. The cost for an adult usually exceeds \$250,000 per year per patient.

Gene therapy is a novel approach to the treatment of genetic diseases. It involves the addition of a normal gene to the cells of a patient with an inherited defect in the corresponding gene. Bone marrow cells are an important target cell for gene transfer in Gaucher disease because MØ are derived from the bone marrow. In the proposed approach to treatment, cells that can make the bone marrow would be collected from the patient, genetically corrected by inserting the normal gene, and then re-colonized in the patient's bone marrow. This is called gene transfer and autologous BMT. It avoids the immunologic problems of graft rejection and graft-versus-host disease, which occur with high frequency in bone marrow transplantations from a donor.

To be successful, gene therapy requires high efficiency gene transfer into cells, followed by the sustained activity of the transferred gene to do the job of making the enzyme. The most efficient way to transfer and express genes in mammalian cells is by the use of modified viruses acting as carriers of genes. These are called vectors. The most often used and most completely studied vectors are derived from retroviruses. Retroviral vectors use the natural ability of these life forms to infect cells and integrate their genetic material into the target cell's chromosomes. By this action, these vectors provide cells with potentially therapeutic genes. Retroviral vectors can be rendered unable to multiply themselves and still retain the ability to transfer genes into cells of many different types leading to stable, intact residence in the host cell DNA.

Correction of the genetic defect in bone marrow producing cells could result in the life-long production of enzymatically competent MØ. This should be therapeutic for patients with GD.

We have isolated the gene for glucocerebrosidase(GC) and demonstrated that transfer of the gene reversed the enzyme deficiency in the cells of patients with Gaucher disease. We have shown in bone marrow transplantation studies in mice that a retroviral vector can efficiently transfer the GC gene to primitive cells in bone marrow. Moreover, the human gene produced the GC enzyme in macrophages for the life of the mice. Furthermore, we have shown that transfer of the GC gene to macrophages obtained from the blood of Gaucher patients completely corrected the deficiency of the enzyme (GC) in this important cell. We also have transferred the normal human GC gene to cells from human blood enriched for bone marrow producing cells (CD34⁺) capable of reconstituting the bone marrow. The transduced cells expressed 2-4 times the amount of GC enzyme normally present in these cells. Recent results reveal that the CD34⁺ cells from a Gaucher patient are also corrected to 2-4 times the normal amount of enzyme. This is an important advance because the correction occurs in a cell which can re-establish the entire bone marrow including macrophages. The success of these studies permit consideration of clinical trials of gene therapy for patients with Gaucher disease.