Prospects for Research in Hematologic Disorders

Sickle Cell Disease and Thalassemia

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Sickle cell anemia and thalassemia constitute the most common genetic diseases in the world. Affected patients carry a heavy burden of morbidity and early mortality. With improved understanding of the pathophysiology and molecular basis of these diseases, treatment is evolving from management of symptoms to more effective strategies that aim to modify diseased red blood cells or replace them with normal cells. Available treatment options include red blood cell transfusions, pharmacologic interventions to increase fetal hemoglobin levels, and stem cell transplantation. Improvements in these approaches or the development of means to replace defective genes with normal ones using techniques of gene transfer offer hope for the future.

Recent Advances

The past 25 years have witnessed real advances in the management of hemoglobinopathies. In sickle cell disease, prevention of overwhelming bacterial infection by immunizations and prophylactic antibiotics have almost eliminated deaths from these infections during infancy. Treatment is evolving from management of symptoms to strategies that modify diseased red blood cells or replace them with normal cells. High fetal hemoglobin levels reduce the clinical severity of sickle cell disease or thalassemia, sparking a search for drugs that increase fetal hemoglobin levels in red blood cells. One, hydroxyurea, is a mainstay of treatment in sickle cell disease and reduces the frequency of hospitalization in some patients. Monthly red blood cell transfusions can prevent most disease-associated morbidity, but transfusion complications such as iron overload, which injures heart, liver, and endocrine tissues, remain a challenge. If used consistently, an iron chelator, deferoxamine, can reduce the body iron burden to safe levels; however, the drug is expensive and its administration is so cumbersome that good compliance is uncommon. Both sickle cell disease and thalassemia can be cured by stem cell transplantation, but transplant-associated complications, expense, and a paucity of suitable donors have limited its application.

Lack of a widely available cure underscores the importance of disease prevention. DNA testing of fetal blood or tissues to identify sickle cell disease or thalassemia allows elective termination of affected pregnancies. In Italy and Greece, where thalassemia is the most common genetic disease, prenatal diagnosis has reduced the disease burden to families and to the health care system. A costly and labor-intensive alternative approach, preimplantation diagnosis, relies on in vitro fertilization and DNA testing of embryos to select those free of disease for implantation.
Current Scientific Foundation

Sickle hemoglobin polymerizes when deoxygenated, forming rigid structures that deform red blood cells into sickle shapes that block the flow of blood. The presence of certain nonsickle hemoglobins (ie, fetal hemoglobin) within red blood cells retards the sickling process. Other factors such as red blood cell dehydration or the increased adherence of sickle red blood cells to vascular endothelium initiate occlusive events. Agents that can favorably affect erythrocyte deformability, adherence, hydration, hemoglobin composition, or the polymerization of sickle hemoglobin need to be identified. The availability of transgenic mice that have red blood cells largely filled with human sickle hemoglobin has made it possible to investigate new agents in an animal model of sickle cell disease. This model is also likely to provide insight into the complex basis for the vasocclusive process and the organ damage that ensues.

Hemolytic anemia in thalassemia is due to unbalanced production of α-globin and β-globin chains. Excess globin chains produced by the nonthalassemic normal allele accumulate within and ultimately destroy red blood cells. A spectrum of genetic defects, ranging from large deletions to point mutations located in regulatory regions or in globin coding sequences, lead to thalassemia, accounting for its remarkable clinical variability. Genetic or environmental factors that reduce the imbalance in globin chain synthesis mitigate clinical severity. For example, agents that increase fetal hemoglobin production may reduce the clinical severity of thalassemia. These agents seem to be less effective in thalassemia than in sickle cell disease, however, and none are in routine clinical use. As in sickle cell disease, transgenic mouse models of thalassemia are useful to the understanding of disease mechanisms and screening for potential therapeutic agents.

Promising Research Initiatives

Current research initiatives in stem cell transplantation focus on reducing the toxicity of pretransplant conditioning regimens and expanding the stem cell donor pool. Under evaluation are stem cells from unrelated adult or cord blood donors and from partially compatible, T-cell–depleted parental or sibling donors. The utility of such donors may be limited by graft failure, graft-vs-host disease, or delayed immune reconstitution unless these problems become manageable. In utero stem cell transplantation in sickle cell disease or thalassemia fetuses identified by prenatal diagnosis has been unsuccessful thus far because donor stem cells are not able to replace enough recipient stem cells.

Recent progress has increased hope for the future of gene therapy for hemoglobinopathies. Although it is possible to introduce intact β-globin genes into murine hematopoietic stem cells using retroviral vectors, efficiency of transfer is low, expression of the transgene suboptimal, and long-term stability limited by gene silencing and position effects. Use of a lentivirus vector derived from human immunodeficiency virus 1 and carrying a large fragment of the human β-globin gene and its locus control region yields better and more stable expression, in the range that would be expected to be therapeutically effective.

Needed Research Advances

To take advantage of the ability of fetal hemoglobin to inhibit the sickling process in sickle cell disease and to replace missing hemoglobin molecules in thalassemia requires greater understanding of how the globin genes are regulated. Such an understanding may lead to the development of pharmacological agents that allow the continued expression of the fetal hemoglobin gene. The ideal treatment of genetic defects is, of course, their full correction. To achieve this, 2 major hurdles need to be overcome. The first is the identification of a stem cell capable of perpetual renewal and differentiation into mature blood cells. Discovery of the conversion of stem cells from the blood to liver and muscle cells, and from the brain to blood cells are most encouraging, and research in this area should receive high priority. The second is the actual correction of genetic defects within the stem cell by introduction of a normal gene into the cell. Harmless vectors should be designed to introduce normal genes efficiently. The inserted genes should also function at an appropriate and adequate level throughout life. One approach is correction of the mutation.
itself by homologous recombination with a piece of DNA that contains the normal sequence. Although feasible, the frequency of successful recombination is currently too low for practical application. Until there is a cure, treatment modalities used for these patients need to be improved. For example, simpler ways of obtaining pathogen-free blood will make transfusions safer. Iron chelators that can be given orally or injected infrequently should encourage patient compliance. Noninvasive imaging techniques that can measure iron accumulation in the liver, heart, and endocrine glands will improve monitoring of chelation therapy.

A frequent complication of sickle cell disease, especially during childhood, is cerebral infarction. A better understanding of the mechanisms that cause adherence of sickle cells to vessel walls and activation of blood clotting may suggest new therapies to prevent damage to the brain and other vital organs.

**Research Prospects and Opportunities**

Although eradication of a genetic disease by manipulating the gene in the germ line is not yet possible, more effective somatic cell treatments will become available. The most desirable form of therapy is to correct defective genes with normal ones in hematopoietic stem cells. Although many barriers remain to be overcome, gene therapy, perhaps involving homologous recombination currently in use in animal models, might be available within the next 25 years. In less developed countries where these diseases are common, the widespread application of gene therapy may be constrained by cost factors. Similar constraints apply to stem cell transplantation, although outpatient transplantation following minimal conditioning may reduce cost. New pharmacological agents to increase fetal hemoglobin levels, increase red blood cell hydration, and directly inhibit the sickling process in vivo are needed. Combination chemotherapy, as in the treatment of cancer, may be more effective than single agents. The pharmacological agents envisioned would need to be administered throughout the lifetime of the patient, so that even if daily cost is small, long-term costs might be equivalent to or even greater than stem cell transplantation or gene therapy.

Until inexpensive and effective curative treatment becomes available, the greatest impact on the burden of hemoglobinopathies worldwide will continue to be their prevention by prenatal diagnosis and elective termination of pregnancies found to be affected. Simplification of techniques for prenatal diagnosis, perhaps using gene chip technology, may make them more widely available. One promising approach is isolation of fetal cells from the maternal circulation, followed by polymerase chain reaction–based DNA diagnosis. This approach must be simplified to keep costs down and to reduce the level of technical expertise required to obtain reliable results.

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**REFERENCES**