Division of Blood Diseases and Resources

Cooley's Anemia: Progress in Biology and Medicine - 1995

NATIONAL INSTITUTES OF HEALTH
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

Cooley's Anemia: Progress in Biology and Medicine - 1995

A review of the status of activities in basic and clinical research in Cooley's anemia and recommendations for the future.

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PREFACE

This report is a noteworthy example of the commonality of interests of the National Heart, Lung, and Blood Institute (NHLBI), the Congress, the biomedical community, the Cooley's Anemia Foundation, and the patients and families who continue to struggle with difficult and sometimes inadequate therapy for Cooley's anemia. This disease is of interest not only because of concern with the clinical problems of patients but also because unique features of the disease provide opportunities for fundamental research into mechanisms relevant to other more common inherited and acquired diseases.

The Institute's interest dates back to 1968, when the first two patients with Cooley's anemia were admitted to the National Institutes of Health (NIH) Clinical Center in our Intramural Research Program. Since then, both the Intramural and Extramural Programs have expanded substantially. In 1980, and again in 1987, in response to congressional interest, the Institute evaluated the state-of-the-art in Cooley's anemia research and patient care. In the interval since the last report, considerable advances have been made in the regulation of gene expression, hematopoietic stem cell biology, gene therapy, bone marrow transplantation, and other areas of clinical research, particularly fetal hemoglobin enhancing drugs and oral iron chelator development. Because these advances are so relevant to the problems underlying Cooley's anemia, it is timely to update this assessment of the status of research and treatment. *Cooley's Anemia: Progress in Biology and Medicine—1995* was prepared by a distinguished group of consultants chaired by Dr. Alan Cohen. We hope it will be of value to those involved directly with the care of patients and serve as a source of ideas to those delving into an understanding of a disease that has thus far eluded satisfactory treatment modalities and a final cure.

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Director
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Executive Summary

Cooley's anemia (also called beta-thalassemia, thalassemia major, or Mediterranean anemia) is a genetic blood disease that results in an inadequate production of hemoglobin, the essential oxygen-carrying substance in blood. This causes severe anemia that begins shortly after birth. As a group, thalassemic disorders are one of the most common genetic diseases worldwide. In the United States, thalassemia mainly affects specific ethnic groups, including people of Mediterranean, Middle Eastern, African, Southeast Asian, Chinese, and Asiatic Indian origin. Individuals affected with Cooley's anemia require frequent and lifelong blood transfusions to sustain life. Because there is no natural means for the body to eliminate iron, the iron contained in the transfused red blood cells builds up over many years and eventually becomes toxic to tissues and organ systems. This excess iron must be removed from Cooley's anemia patients or they may not survive beyond the first decade of life. To accomplish this, patients must use the iron-binding or chelating drug, deferoxamine, that is administered for about 10 hours every day by pumping it through a needle inserted either under the skin or intravenously.

In 1987, a committee of distinguished consultants, responding to a mandate from Congress, prepared a report for the NHLBI. This committee reported on progress in research and medical care for patients with Cooley's anemia and recommended a number of topics for future study. In the 8 years since that report, there has been major progress both in basic research and in the application of that knowledge to the clinical care of patients. Consequently, another working group of distinguished experts performed an intensive review of the status of research and patient care and compiled this new report. In addition to summarizing significant progress, the report provides recommendations for research to improve clinical care of affected patients and, over the long term, to find a cure for the disease. A listing of those involved in the preparation of this most recent review follows this Executive Summary.

Many of the landmark advances in biomedical research can be traced back to basic research in the area of the hemoglobinopathies and especially Cooley's anemia. Research on Cooley's anemia has contributed to a wealth of knowledge in human biology and medicine. Fundamental knowledge about the control of gene expression has resulted from basic research on the thalassemias. Over the years, this research has been applied to abnormal gene function in several more common diseases such as sickle cell disease, diabetes, and cancer. The innovative approaches to treatment of Cooley's anemia patients with blood replacement therapy and iron chelation have been applied to more common

diseases. The knowledge gained from basic research on gene therapy approaches to the treatment of Cooley's anemia and bone marrow and stem cell transplantation will be directly applicable to the treatment and cure of dozens of genetic and acquired hematologic diseases as well as to metabolic, neurologic, oncologic, and other disorders.

Before the introduction of transfusion and chelation therapy, life expectancy for patients with Cooley's anemia was 2 or 3 years. Today, the picture is considerably brighter with some patients living into their 30's and beyond. Survival and quality of life depend upon the absence or control of transfusion complications such as infectious diseases (mainly hepatitis and AIDS) and the adequate removal of excess iron.

In spite of the increased survival and quality of life, the current treatment modalities are inadequate. Iron-chelation therapy is extremely demanding, and many patients experience considerable discomfort from administration of the drug. In fact, the therapy is so burdensome that many adolescents and teenagers have refused to continue. Indeed, the prognosis for these noncompliant patients is quite poor. In addition to the physical and psychological burden of therapy, the financial burden on the family is incredible. The cost of the transfusions and iron-chelation therapy alone is about $30,000 per year per patient, and it has been estimated that the total annual Cooley's anemia-related costs may be as much as $100,000 per year per patient.

The search for an improved chelator continues. A variety of compounds have been produced and examined as potential oral iron-chelating agents, but only one of these is being evaluated in clinical trials: deferiprone (1,2-dimethyl-3-hydroxypyridin-4-one, also known as L1, CP20, and DMHP). A recent prospective evaluation of this orally active chelator has provided evidence that deferiprone induces sustained decreases in body iron to concentrations compatible with the avoidance of the complications of iron overload. Unfortunately, some patients have developed a life-threatening drop in their white blood cell counts (neutropenia or agranulocytosis) during deferiprone therapy. Until the magnitude of this risk of agranulocytosis is determined, deferiprone must be considered an investigational drug restricted to patients unable or unwilling to use deferoxamine. The search for still safer and more effective oral iron chelators must continue.

Progress in our ability to reverse the hemoglobin switch and turn on fetal hemoglobin production has increased in the past decade, giving promise that a new, effective form of therapy for thalassemic patients may soon be available. Three classes of drugs, (1) cytotoxic drugs (chemotherapy-type drugs), (2) hematopoietic growth factors, and (3) fatty acids, have been shown to enhance fetal hemoglobin production, but their effects have been minimal. However, there are encouraging suggestions that the effects of these drugs may be additive or synergistic and, therefore, limited trials using combinations of these agents are encouraged. Further research is necessary to understand more precisely how these drugs induce fetal hemoglobin production, and additional clinical trials are needed to test the effectiveness and safety of these and other new hemoglobin switching agents as they are identified.

The prospects for gene therapy for Cooley's anemia seem very good. However, the complexity of the biological problems that remain to be solved is considerable. These problems include learning how to effectively

insert normal genes into nondividing hematopoietic stem cells, developing better vectors for gene transfer, and clarifying regulatory mechanisms that control the function of the transplanted normal genes. With continued support for these research efforts, the goal of a nontoxic, curative therapy for Cooley's anemia should be achievable in the not too distant future.

Bone marrow transplantation for Cooley's anemia has been highly successful for a subset of patients who have undergone this procedure. However, the majority of patients are either not suitable candidates for a transplant or they do not have a suitably matched marrow donor if they are otherwise eligible. Current research efforts in stem cell biology and immunology and ongoing studies of mismatched donor transplants and *in utero* transplants will advance the field of stem cell transplantation for many diseases and will widen the application of this therapy for Cooley's anemia.

Twenty years ago, Cooley's anemia patients had little hope of living beyond adolescence, and decisions about advanced education, vocation, marriage, and childbearing were minor or unneeded. Now that patients are living longer, they are encountering problems related to these issues. New challenges arise as more patients face psychosocial, fiscal, and logistical problems in managing their lives. The expenses of complex and chronic medical care for Cooley's anemia patients are extensive and pose a financial burden for them and for their families. There is a need for new psychosocial resources, trained people, and information.

Without a doubt, the remarkable improvement in the prognosis for Cooley's anemia patients in the past 20 years can be traced back to research advances that emerged as a result of specific initiatives implemented by the NHLBI. This marvelous progress would not have been possible without the leadership role taken by the NHLBI. The research momentum that has developed over the past several years needs to continue. The Institute is urged to continue its leadership role until improved treatments and a cure are found. It is hoped that resources will become available for the continuing implementation of initiatives and for providing increased support for research in the following important areas covering a spectrum of basic, clinical, and psychosocial research. Each of these recommendations is described in greater detail in Chapter V—Recommendations for Research.

- Develop safe and effective orally active iron-chelating agents.
- Develop a consensus regarding the optimal management of thalassemia intermedia.
- Develop approaches to the accurate, noninvasive assessment of body iron burden.
- Develop safe and effective therapies to enhance fetal hemoglobin production.
- Support stem cell biology and transplantation research.
- Expand support for gene therapy research.
- Enhance the safety of the blood supply provided to patients with Cooley's anemia.
- Develop safe and effective treatment of viral hepatitis.
- Investigate the psychosocial needs related to increased life expectancy.

- Adapt current counseling programs and written materials for multicultural application.
- Develop new approaches to improving compliance with iron-chelation therapy, particularly among teenagers.
- Investigate the psychosocial impact of hormone therapy in adolescents and young adults.
- Establish a network of medical centers to enable rapid testing of new clinical modalities.

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Chapter I

Background and Definition of the Disease

A. INTRODUCTION

Hemoglobin, the red-colored protein in the red blood cells, consists of an alpha (α)-globin and a beta (β)-globin portion. The thalassemic disorders are a group of genetic blood diseases in which the decreased production of the alpha- or beta-globin component of hemoglobin causes anemia, which begins in early childhood and lasts throughout life. Thalassemia major refers to those forms of thalassemia in which the anemia is so severe that the affected individual requires regular blood transfusions to sustain life. Thalassemia intermedia describes a form of thalassemia in which the anemia is moderately severe and may not warrant the use of regular transfusion therapy. However, there is a wide variation in the clinical severity of thalassemia intermedia. A more complete description can be found in chapters III and V. Thalassemia minor, or thalassemia trait, refers to the carrier state of thalassemia in which the anemia is minimal or mild and not accompanied by symptoms.

The thalassemia syndromes are further defined by the portion of the hemoglobin molecule that is deficient. For example, the most common clinically important thalassemic disorder in the United States is beta-thalassemia major in which beta-globin is absent or markedly diminished; the deficit in beta-globin production and the relative excess of alpha-globin contribute to ineffective red blood cell formation that leads to the severe anemia that requires regular blood transfusions. In alpha-thalassemia, on the other hand, the amount of alpha-globin is reduced. Severe deficits in alpha-globin production may also cause transfusion-dependent anemias. Although most thalassemia disorders are due to the underproduction of a structurally normal hemoglobin molecule, some are due to structural alterations of either the alpha-globin (e.g., hemoglobin Constant Spring) or the beta-globin (e.g., hemoglobin E) that result in reduced amounts of the globin component.

Thalassemia major is also known as Cooley's anemia, named after the Detroit pediatrician who published the first clinical description of the disease in 1925. This eponym was later associated with beta-thalassemia, the common form of the disease in the large immigrant groups from Italy and Greece. As the pathophysiology and molecular biology of thalassemia have unfolded, investigators have recognized that different types of thalassemia may have similar clinical characteristics. Thus, transfusion-dependent thalassemia, whether due to alpha-thalassemia, beta-thalassemia, or a combination of different types of thalassemia, is often called Cooley's anemia. In some medical centers, a large proportion of the patients with Cooley's anemia may have alpha-thalassemia syndromes, commonly

found in individuals of Asian ancestry, although in other centers most patients are of Italian or Greek ancestry and their Cooley's anemia is due to beta-thalassemia.

Thalassemia major occurs when a baby born to parents who are both carriers for thalassemia inherits the abnormal gene from both the mother and the father. Figure I-1 illustrates the possible genetic outcomes for each pregnancy if both parents have the beta-thalassemia trait. Each baby born to such a couple has a 25 percent chance of having thalassemia major (two beta-thalassemia genes), a 50 percent chance of having thalassemia trait (one beta-thalassemia gene and one normal beta-globin gene), and a 25 percent chance of having no thalassemia abnormality (two normal beta-globin genes). Parents who are carriers for two different types of thalassemia such as the beta-thalassemia trait and the hemoglobin E trait may also have a baby with Cooley's anemia. The inheritance of alpha-thalassemia is more complicated than beta-thalassemia

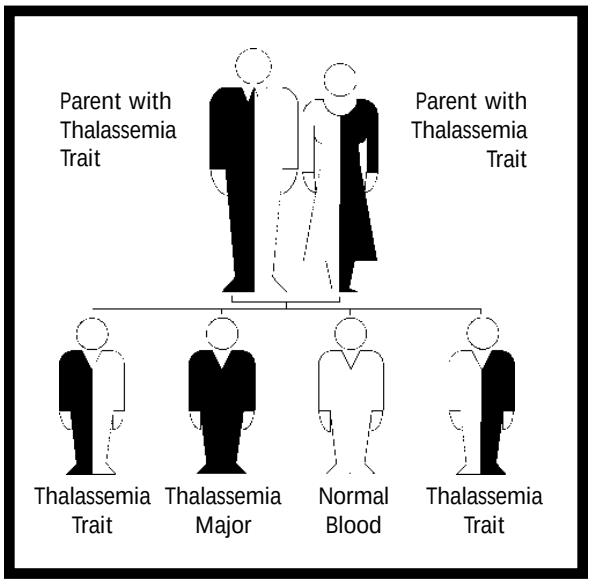

Figure I-1
Inheritance patterns if both parents have a Cooley's anemia gene.

because each person normally has four alpha-globin genes, two on each chromosome. Carriers of alpha-thalassemia have one or two defective alpha-globin genes. Inheritance of three defective alpha-globin genes (one from one parent and two from the other) leaves the infant with only one normal alpha-globin gene instead of four, a condition known as hemoglobin H disease. Some patients with hemoglobin H disease require regular red blood cell transfusions because of severe anemia. Inheritance of four defective alpha-globin genes, two from each parent, causes severe anemia and, in most cases, death of the unborn child.

B. CHANGING PATTERN OF CARE

The remarkable progress that has occurred in the treatment of Cooley's anemia during the past 30 years is readily illustrated by a comparison of the lives of patients born at the beginning and near the end of this period. The child diagnosed with Cooley's anemia in 1960 received blood transfusions only after becoming very pale and tired (figure I-2). Because the hemoglobin levels were very low (e.g., 4 g/dL) at the time of transfusion, the patient was admitted to the hospital for at least 24 hours to receive several units of blood. The severe anemia and the accompanying expansion of the bone marrow left the patient thin, weak, susceptible to fractures, and with characteristic distortion of the skull and facial bones. Massive enlargement of the liver and spleen caused the abdomen to protrude, adding to the sickly feeling and appearance of the patient. The severe anemia before each transfusion often meant that the patient with Cooley's anemia was unable to attend school for weeks at a time. Many patients died in early childhood because of congestive heart failure due to severe anemia. The

Figure I-2
Child with Cooley's anemia in the 1960's.

others had stunted growth, failed to develop sexually, and died during the second decade of life from cardiac and liver damage caused by excessive iron that accumulated from the blood transfusions.

The child with Cooley's anemia in the 1990's has a strikingly different course and prognosis. These improvements are directly derived from advances in basic and clinical research. Blood transfusions are administered in an outpatient setting every 2 to 4 weeks to keep the hemoglobin higher than 9 to 10 g/dL. This hypertransfusion program alleviates disfigurement and allows the patient to engage in most normal activities (figure I-3). The higher hemoglobin level and the relatively brief time (4 to 6 hours) required for the transfusion of blood, result in a sense of well being and permit schooling or work to continue with comparatively less interruption. Regular administration of the iron-chelating drug deferoxamine prevents the accumulation of excessive iron so that growth and sexual maturation are normal or almost normal, and iron-induced

Figure I-3
Child with Cooley's anemia in the 1990's.

damage to vital organs is prevented. Regularly transfused and well-chelated patients with Cooley's anemia in the 1990's are undistinguishable from their peers, are productive members of the workforce, and can anticipate a much improved lifespan. Simultaneously, the improved survival of patients with Cooley's anemia has transformed regular care into an ongoing clinical research project as new issues such as fertility, long-term effects of hepatitis or other blood-borne diseases, optimal levels of iron, and social adaptation arise regularly.

Patients with Cooley's anemia who have a brother or sister with the same tissue type have an alternative to transfusion and chelation therapy, namely bone marrow

transplantation. Successful replacement of the bone marrow with that from a human leukocyte antigen (HLA)-identical brother or sister results in the production of normal red blood cells and the resolution of the anemia, often referred to as thalassemia-free survival. Bone marrow transplantation is an option that has been chosen by hundreds of patients and families throughout the world. However, most patients with Cooley's anemia cannot be treated with bone marrow transplantation because of the lack of a suitable donor. Research into the use of alternative donors holds the promise of wider application of this approach.

C. NEED FOR NEW TREATMENTS

These major advances in the treatment of Cooley's anemia have carried a personal and financial cost. Successful iron chelation therapy requires an 10-hour nightly infusion of deferoxamine (figure I-4). This method of administration is often associated with pain at the infusion site, and the drug has also been responsible for side effects in a few patients. Blood transfusions, although safer than ever as a result of improved donor selection and testing for various infectious agents, still may transmit serious viral and bacterial diseases, including hepatitis and AIDS. This risk is magnified for the patient with Cooley's anemia who will receive more than 1,500 units of blood in his or her lifetime. Both chelation and transfusion therapy are very expensive; the annual costs for these items alone usually exceed $30,000. It has been estimated that the total annual Cooley's anemia-related medical costs may be as high as $100,000 for each patient. Those who have no insurance face overwhelming financial problems and patients with insurance may be left with large copayments.

Figure I-4
Child undergoing iron-chelation therapy.

Bone marrow transplantation is currently feasible for only a minority of patients with Cooley's anemia. Moreover, patients who have unsuccessful engraftment may be left with their original thalassemia, while others undergoing this procedure may die or develop serious complications. Bone marrow transplantation costs more than $100,000, and many insurance companies consider it experimental (for Cooley's anemia) and may not provide benefits. These concerns explain why patients with Cooley's anemia in the 1990's, despite the many advances in medical care, seek better options for treatment and eagerly await new curative therapies.

The triumphs of molecular medicine that have occurred in the past 15 years and the intensive basic and clinical research investigations presently under way are described in the following chapters. These are the keys not only to the successful treatment of Cooley's anemia but also to the understanding and potential cure of all inherited diseases.

CHAPTER II

DEMOGRAPHICS AND MAGNITUDE OF THE PROBLEM

A. NORTH AMERICA

Fifteen years ago, national surveys using several different methodologies estimated that there were fewer than 1,000 patients with Cooley's anemia in the United States. In 1993, a questionnaire was sent to 43 medical centers in the United States and Canada. These included all centers known to have significant numbers of Cooley's anemia patients. Information was requested about current patients with homozygous beta thalassemia. A total of 518 patients were reported; 88 percent of whom had thalassemia major (transfusion-dependent Cooley's anemia) and 12 percent had thalassemia intermedia.

The age distribution of the 443 patients with thalassemia major is shown on figure II-1. The mean age was 16.2 years. Fewer than 1 percent of the thalassemia major patients were older than 41 years.

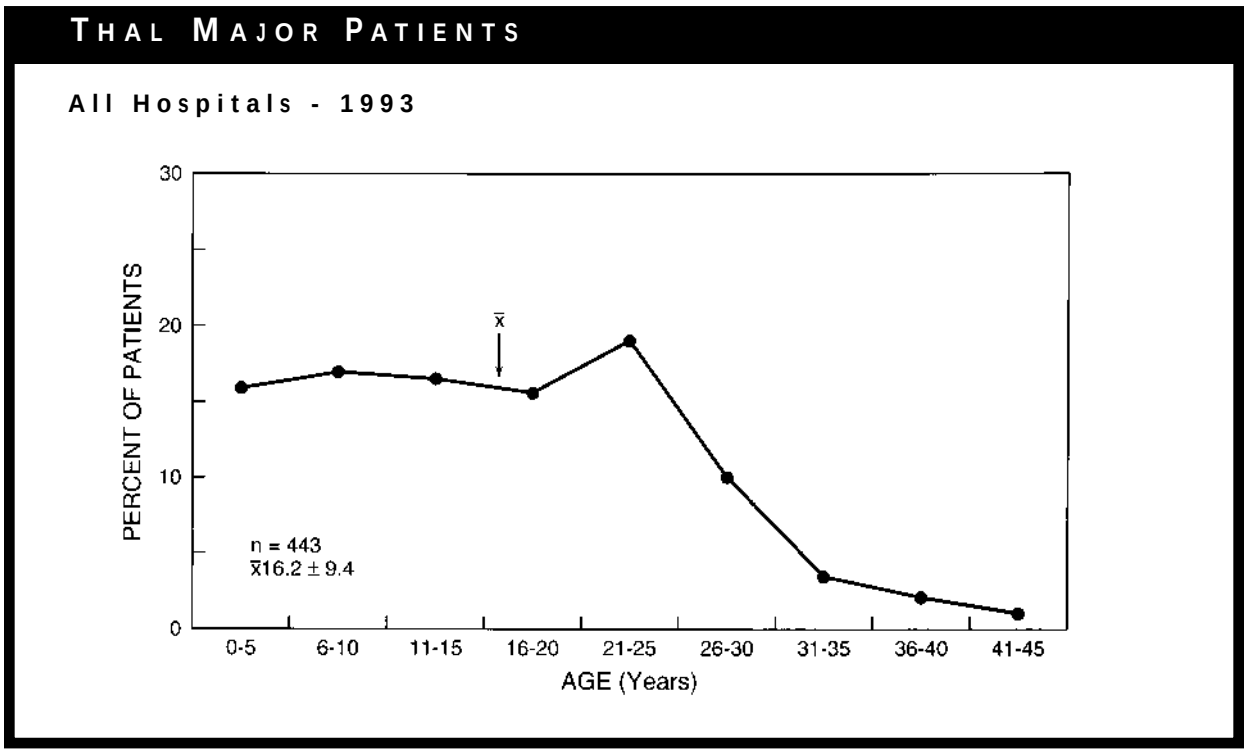

Figure II-1

Thalassemia major patients reported by 43 North American medical centers.

[Reproduced by permission of *Pediatrics*, Vol. 97, 1996]

Many different ethnic backgrounds were represented, but 62 percent were of Italian/Greek backgrounds (table II-1). Within the United States, the great majority of patients resided in the Northeast (table II-2).

It is likely that this survey encompassed the great majority of North American patients. Although some patients may be followed in adult hematology centers, their total number is doubtless quite small and most pediatric hematology centers continue to treat or maintain contact with adult patients. Asymptomatic thalassemia intermedia patients might also not be represented, but again, their numbers are small. Therefore, similarly to what was found 15 years ago, there are fewer than 1,000, and likely fewer than 750, homozygous beta-thalassemia patients in North America today.

Although the relatively few patients make thalassemia major an "orphan disease" in North America, the impact of the disease is great in several ethnic communities. Furthermore, the resources that are necessary to care for these patients, including the need for large amounts of blood, give thalassemia an importance far beyond the numbers of patients alone. Studies of these patients have contributed to major scientific advances in the understanding of many human diseases.

When the current thalassemia major patients were separated by their ethnic backgrounds, striking differences in age distribution were apparent (figure II-2). Two hundred and seventy-one thalassemia major patients of Italian/Greek ancestry had a low proportion of younger patients, and

Table II-1

Ethnicity of Homozygous B-thalassemia Patients (Major and Intermedia)

	Number	Percent
Italian	229	44.2
Greek	92	17.8
Indian/Pakistani	56	10.8
Middle Eastern	47	9.0
Chinese	43	8.3
African American	22	4.2
Southeast Asian	19	3.7
Other	10	1.9
	518	99.9

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Table II-2

Geographic Location of Thalassemia Major Patients

Hospital Location	Number	Percent
Canada (Toronto, Montreal)	116	26.0
New York City (Cornell, Columbia, NYU, Mt. Sinai)	88	20.0
Other New York State Long Island, Syracuse Rochester, Westchester	54	12.0
California (Oakland, Los Angeles)	52	11.7
North East Boston, New Haven, Providence	44	10.0
Philadelphia	30	6.7
Chicago	15	3.5
All Other Locations	44	10.0
TOTAL	443	99.9

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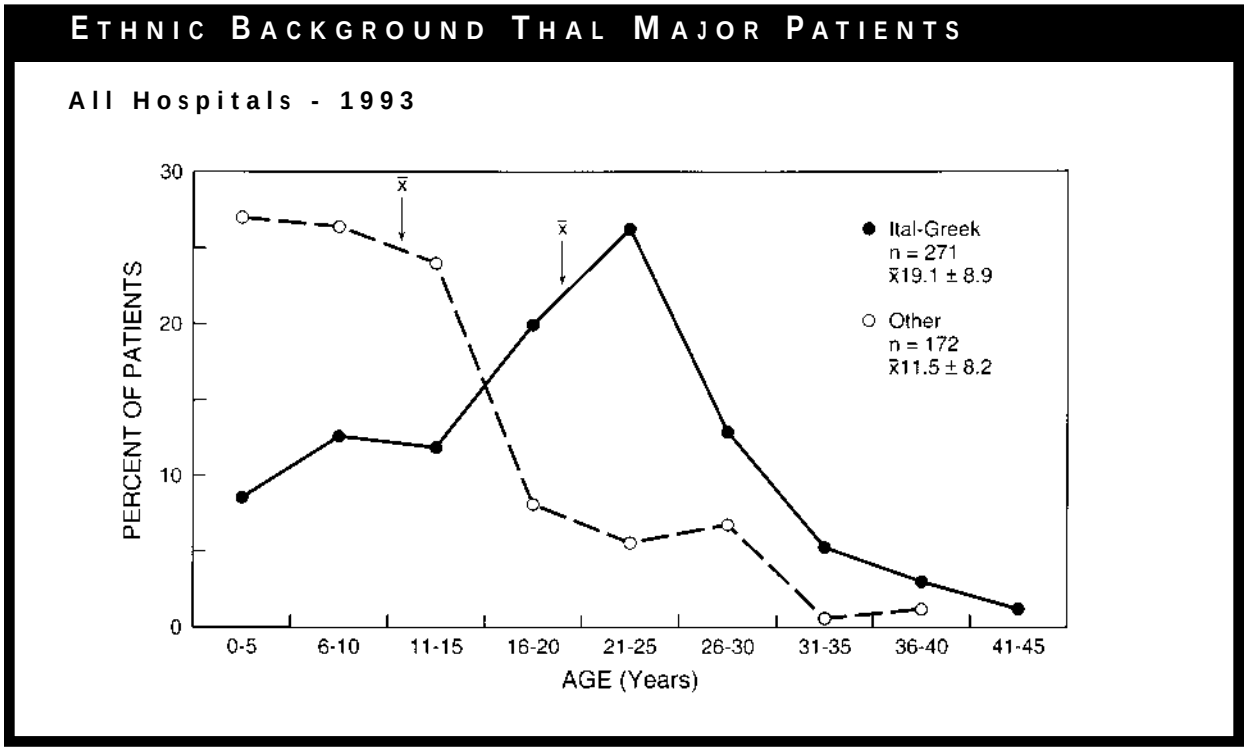

Figure II-2

Ages of thalassemia major patients and their ethnicity. Ages of Italian/Greek thalassemia major patients are contrasted with patients of all other ethnicities.

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66 percent were older than 16 years of age. The mean age of the Italian/Greek patients was 19.1 ± 8.9 years. In contrast, 172 thalassemia major patients from other ethnic groups (Middle Eastern: Turkish, Arabic, Irani; Indian-Pakistani; Chinese; and Southeast Asian) were considerably younger. Their mean age was 11.5 ± 8.2 years, and 77 percent were younger than age 15.

Census data over the past 20 years are shown on table II-3. There have been large numbers of legal immigrants into the United States from the ethnic groups that contributed heavily to today's young thalassemia major cohort.

Progress in the clinical management of thalassemia major can be assessed by examining the ages of thalassemia major patients being treated in 12 North American thalassemia centers over the past 20 years: in 1973, 1985, and 1993 (figure II-3). In 1973, the 12 centers were following 243 thalassemia major patients whose mean age was 11.4 ± 6.7 years. In 1985, 303 patients had a mean age of 14.2 ± 7.3 years. In 1993, 301 patients had a mean age of 16.1 ± 9.1 years. Similar to the data from all 43 hospitals, most of the non-Greek/Italian thalassemia major patients in the 12 centers were younger than age 15.

These data indicate increasing survival of thalassemia major patients. In 1973, only 16 percent of thalassemia major patients were older than age 20; in 1993, this percentage had doubled to 33 percent. In addition, the

Table II-3

Thalassemia Intermedia and Ethnicity

	Total Patients	Thalassemia Major	Thalassemia Intermedia	Percent Thalassemia Intermedia
Italian	229	198	31	13.5
Greek	92	78	14	15.2
Indian/Pakistani	56	52	4	7.1
Middle Eastern	47	41	6	12.8
Chinese	43	41	2	4.6
African American	22	6	16	72.7
Southeast Asian	19	19	0	0
Other	10	8	2	20
	518	443	75	

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age at which the sharp falloff in the numbers of patients occurred was a decade later in 1995 compared with 1973 (figure II-3). Despite this improvement, there are still only a few current thalassemia major patients who are older than age 35. This probably reflects the fact that effective chelation therapy has been widely used only for the past 10-15 years. Many of the current older thalassemia major patients began effective chelation therapy after they were age 15, when many patients had already developed significant, nonreversible, iron-induced cardiac damage. In addition, noncompliance with deferoxamine chelation therapy is common.

Many of the present thalassemia major patients who are ages 15 to 20 have been effectively chelated since early childhood and many of them have normal cardiac and endocrine function. It is likely that these patients will remain healthy and survive into middle life, or even beyond, if they continue a chelation program.

Summary

A substantial decrease in the number of new thalassemia major patients was observed between 1973 and 1985 at 12 North American thalassemia centers, indicating a decreased frequency of thalassemia major births in the Italian/Greek communities in which screening and genetic counseling initiatives have occurred. There has been no further decrease in the numbers of new thalassemia major patients between 1985 and 1993. This is primarily a consequence of the immigration of non-Mediterranean ethnic groups that have a relatively high prevalence of thalassemia genes. It can be expected that, as newly immigrated families become integrated into the North American

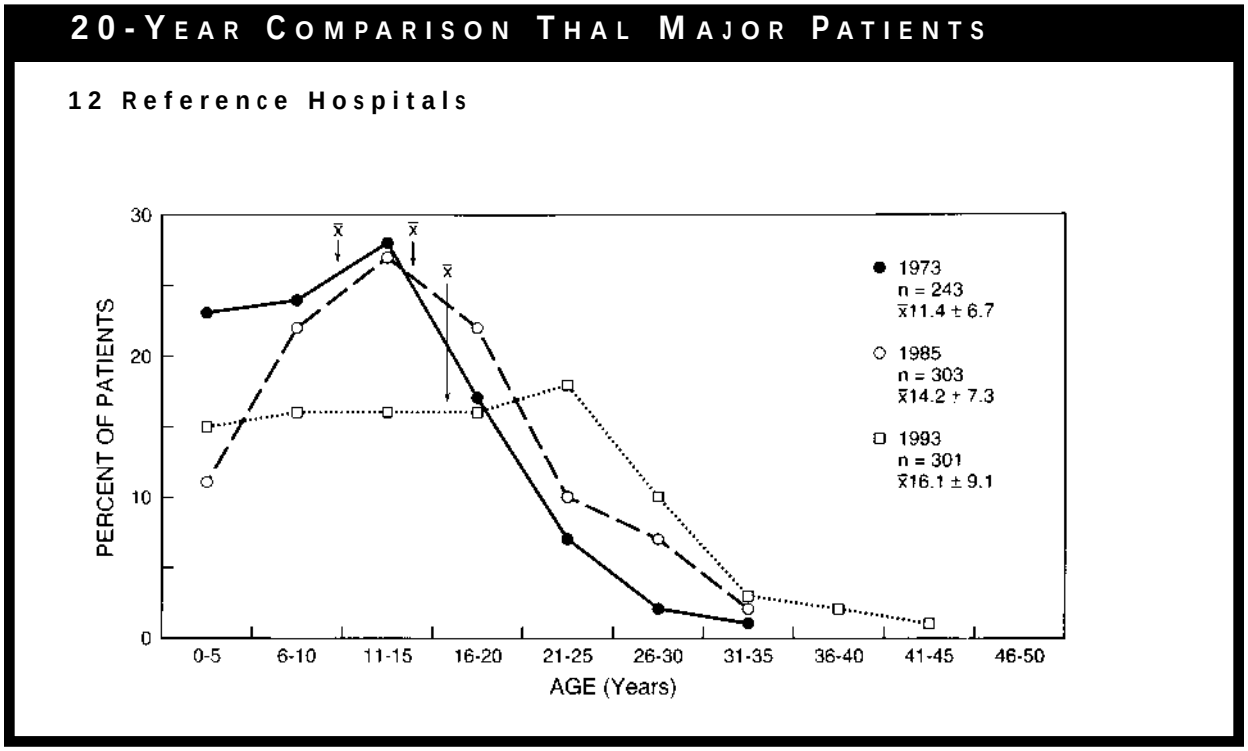

Figure II-3

Age of thalassemia major patients reported from the 12 reference hospitals in 1973, 1985, and 1993.

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medical system, they will become aware of the possibilities of carrier screening, genetic counseling, and prenatal diagnosis. This should result in fewer births of children with thalassemia major in these groups in the future and a further reduction of new cases. However, to accelerate this process, it will be important to develop linguistically and culturally appropriate educational and genetic counseling initiatives directed specifically at these groups.

The increasing ages of thalassemia major patients in North America today has evoked many new problems. Sexuality, marriage, and even reproduction are now important issues for thalassemia major patients. Previously unrecognized acute and chronic medical conditions are being reported. Economic problems are nearly universal for these patients. Older Cooley's anemia patients, no longer eligible for coverage under their parent's insurance, are having difficulty obtaining their own health insurance because of their preexisting health condition.

As fewer Cooley's anemia patients are born in the future, and, as their survival continues to improve because of treatment, Cooley's anemia is no longer an exclusively pediatric problem. Increasingly, it is becoming a disease of young adulthood and beyond.

B. WORLDWIDE DATA

A wide range of different mutations can lead to a hemoglobin disorder. The major hemoglobin disorders are generally grouped into two classes, the thalassemias and the sickling disorders (table II-4).

Table II-4

The Main Hemoglobin Disorders

Thalassemias	Sickling Disorders
β-thalassemias	Sickle cell anemia
β-thalassemia major	HbSS disease
β-thalassemia intermedia	HbSC disease
HbE/β-thalassemia	HbS β-thalassemia
α-thalassemias	
α-thalassemia hydrops fetalis	
HbH disease	

Frequency

The World Health Organization (WHO) estimates of the global prevalence of carriers and the global birth prevalence of hemoglobin disorders are summarized in table II-5. These data have been updated using population figures from the *1991 United Nations Demographic Yearbook* (WHO 1994: Guidelines for control of haemoglobin disorders; Unpublished document WHO/DHP/HB/GL/94.1; obtainable free of charge from the Hereditary Diseases Programme, WHO, Geneva, Switzerland).

Table II-5 shows that globally at least 5 percent of adults carry a hemoglobin disorder. Because the populations in which the disorders are most common also have relatively high birthrates, the proportion of carriers in the global human population is increasing. At present, at least 6.5 percent of the children worldwide are carriers, and WHO projections suggest that when the global population finally stabilizes during the next century at least 8 percent of the global population will carry a hemoglobin disorder. At present, it is estimated that 2.9 percent of adults carry a thalassemia mutation and 2.3 percent a sickling mutation (table II-5).

Population Migrations

The increasing rate of long-distance population migration in the second half of this century has introduced hemoglobin disorders to many nonendemic areas such as northern Europe and Australia. These population movements also have changed the pattern of syndromes that occur. For example, alpha-thalassemia hydrops fetalis is the most common genetic problem among the southern Chinese, and hemoglobin E/beta-thalassemia is the most common hemoglobin disorder among the Thais, Cambodians, Burmese, and Bengalis. As a result of migration from Southeast Asia and south Asia, these previously very uncommon syndromes are now increasingly common in Europe and North America.

A recent report from Europe shows that at least 5 percent of the population and 9 percent of births in northwest Europe are in population groups at risk for the hemoglobin disorders. Migrations related to each country's colonial and trading history have led to different mixtures of population groups and, hence, of hemoglobin disorders. Prenatal diagnosis is available throughout Europe, but the public health approach to population screening for carriers varies greatly among countries.

The study showed that more patients with major hemoglobin disorders are now born annually in northern than in southern Europe. Several factors are responsible for this rather surprising difference. (1) Several groups at particularly high risk have migrated to northern European countries (Sub-Saharan Africans at high risk of sickle cell disease and Cypriots and Pakistanis at high risk of beta-thalassemia). (2) There is a preponderance of sickle cell disorders in the north, and many affected individuals

Table II-5

Carrier Numbers and Births

(Increasing Prevalence of Carriers)

GLOBAL SUMMARY: CARRIERS **DATA COMPILED FOR NIH**

		PERCENT OF POPULATION CARRYING					
Percentage	**Population Millions**	**HbS**	**HbC**	**β Th**	**HbE**	**α0 Th**	**TOTAL**
All Africa	664	10	3	1	—	—	14
All Americas	734.2	1.4	0.4	0.8	—	—	2.4
All Asia	3,143	0.6	—	2.0	1.7	0.4	4.7
All Europe	633	0.12	0.01	1.12	—	—	0.05
Oceana	27	-—	—-	0.8	1	—	—
WORLD	**5,202**	**1.86**	**0.39**	**1.61**	**1.01**	**0.24**	**5.11**

	THOUSANDS OF CARRIERS					
NUMBERS	**HbS**	**HbC**	**β Th**	**HbE**	**α0 Th**	**TOTAL**
All Africa	67,412	17,469	7,689	—	—	92,570
All Americas	10,100	2,579	5,893	—	—	18,572
All Asia	18,514	—	62,653	52,444	12,724	146,335
All Europe	728	48	7,084	—	—	7,861
Oceana	—	—	215	159	—	374
WORLD	**96,754**	**20,095**	**83,535**	**52,602**	**12,724**	**265,711**

ANNUAL BIRTHS OF CARRIERS

			CARRIERS BORN/YEAR, THOUSANDS					
	Births Per 1000	**Births Per year (thousands)**	**HbS**	**HbC**	**β Th**	**HbE**	**α0 Th**	**TOTAL**
All Africa	44.8	29,786	3,186.0	828.4	326.7	—	—	4,341.1
All Americas	24.0	17,606	254.5	65.9	134.3	—	—	454.8
All Asia	26.6	83,489	594.0	—	1,818.7	1,527.8	413.2	4,353.7
All Europe	17.5	11,051	17.0	0.1	117.3	—	—	134.4
Oceana	19.5	524	—	—	0.30	0.24	—	0.54
TOTAL	**27.4**	**142,456**	**4,051.6**	**894.4**	**2,397.3**	**1,528**	**413**	**9,284**
Percent of Babies			2.84	0.63	1.68	1.07	0.29	6.52

DIFFERENCE BETWEEN CHILDREN AND ADULTS

	PERCENT CARRYING					
	HbS	**HbC**	**β Th**	**HbE**	**α0 Th**	**TOTAL**
Adults	1.86	0.39	1.61	1.01	0.24	5.11
Newborns	2.84	0.63	1.68	1.07	0.29	6.52

Table II-5 (continued)

CHANGING PROPORTIONS OF SICKLE AND THALASSEMIA GENES

	ADULTS			NEWBORNS		
Type of Gene	Carriers Thousands	Percent of Traits	Percent of Adults	Carriers Born/Yr Thousands	Percent of Traits	Percent of Newborns
Sickling	116,850	43.98	2.25	4,946	53.27	3.47
Thalassemia	148,861	56.02	2.86	4,338	46.73	3.05
TOTAL	265,711	100	5.11	9,284	100	6.52

survive into their fifth or sixth decade. (3) There is a lower uptake rate of prenatal diagnosis for sickle cell disorders than for thalassemia. (4) Preventive approaches are more fully developed in southern European countries where thalassemia is perceived as an important public health problem than in northern Europe where the hemoglobin disorders affect mainly ethnic minorities.

Population Mixing

The mixing of populations with and without a high prevalence of hemoglobinopathy carriers ultimately, through intermarriage, leads to a major reduction in the birthrate of affected children. This has happened in the United States as many offspring of Mediterranean parents choose a partner with a different background. However, when populations mix, the genes are scattered more widely and an increasing proportion of couples who have no obvious indication of Mediterranean or Asian ancestry are at risk of having affected children. Such couples are at high risk of being missed by the screening programs and of having an affected child unexpectedly. Thus, with time, the indications for carrier screening become more, rather than less, extensive. In the United Kingdom, for example, it is now recommended that in areas with a high proportion of groups at risk, screening should be offered for all pregnant women and newborns rather than being limited to mothers and babies in obvious risk groups.

Prevention of Thalassemia Through Population Screening and Genetic Counseling

Couples at risk of having children with hemoglobin disorders can be identified by blood tests and informed of their one in four chance of an affected child in each pregnancy before they have children. More than 80 percent of counseled couples at risk for having a child with a serious thalassemia syndrome request prenatal diagnosis, and more than 90 percent of parents of an affected fetus request termination of the pregnancy (Br Med J 1980; 2:737). The vast majority of such couples then try again to conceive a healthy child. Because the uptake of prenatal diagnosis and selective abortion is very high (80 percent or more in most populations at risk), programs of population screening and genetic counseling can have a major impact on the birth

rate of affected children. For example, there have been almost no new births of affected children in Cyprus since 1986, and the affected birth rate has fallen to about 20 percent of expectation in the whole of mainland Italy and Greece (figure II-4). The lower fall in northern European countries is primarily due to failure to adequately inform and screen the diverse groups at risk rather than the groups' reluctance to make use of prenatal diagnosis.

The fall in birthrate following genetic counseling is not only due to prenatal diagnosis. Statistics from Cyprus show that about 3 percent of the fall is due to the separation of engaged couples who find they are both carriers, 20 percent is due to limitation of further reproduction by couples at risk once they have two or three healthy children, and about 75 percent is due to selective abortion of affected fetuses and replacement with healthy children.

The introduction of first trimester prenatal diagnosis by chorionic villus sampling and DNA analysis represented a major advance in the prevention of genetic disorders. As a result, prenatal diagnosis (which was initially limited to North America, Europe, and Australia) has spread to many other areas, including the Caribbean area; Turkey; Azerbaijan; Iran; Egypt; Bahrain; Pakistan; India; Singapore; Thailand; and China, including Hong Kong and Taiwan.

The figures for global annual births of carriers given in table II-5 are used in table II-6 as a basis for calculating the "equilibrium" number of living patients one generation after worldwide introduction of basic modern management, leading to present survival

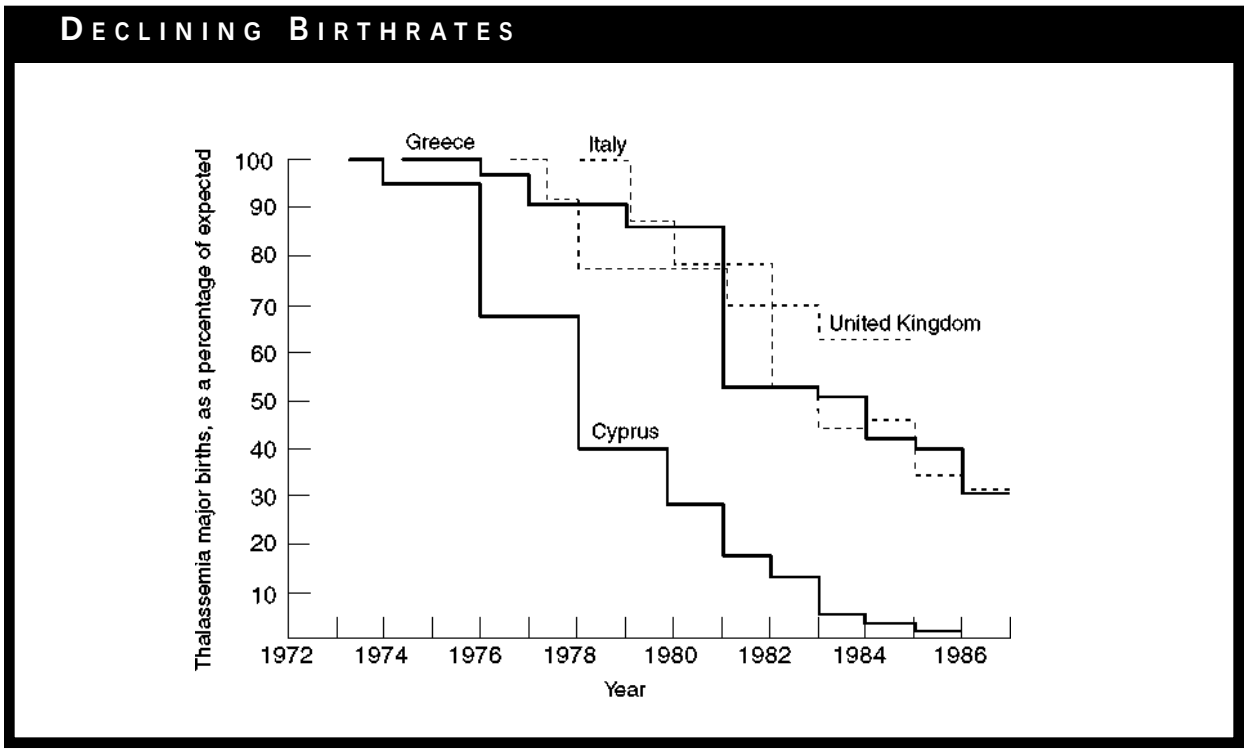

Figure II-4

Fall in the birth incidence of thalassemia major in countries with thalassemia control programs.

Source: World Health Organization

rates. If all patients were treated, the total number under treatment would be about 13 million. Of these, about 2.5 million would have a thalassemia syndrome and about 10.5 million a sickling syndrome. Of course, if the treatment became good enough to permit normal survival, the global prevalence of the hemoglobin disorders would be the same as the birth prevalence, about 2 per 1,000 of the world population.

Ultimately, the entire population needs to be informed of carrier status, but this aim must be approached gradually. Hence, it is reasonable to select a cohort for screening—e.g., pregnant women. They are usually easily accessible to the health system, and information on reproductive risk is of immediate relevance to them. The basic annual requirement for population screening is the annual number of births in populations at risk (about one-half of all births, i.e., about 70 million per year globally). The minimum annual requirement for carrier counseling is the number of pregnant carriers per year. This is one-half the annual number of births of carriers (table II-5), 4.6 million women annually in global terms.

The need for counseling at-risk couples and prenatal diagnosis can be calculated from the estimated affected birthrate, multiplied by four (the number of pregnancies at risk equals four times the number of affected births). The global figure is about 1.4 million at-risk couples per year. The figures show that research on simple and effective approaches to diagnosis and management and prevention of hemoglobin disorders will be needed well into the next century. The global problem will be solved only as a result of research conducted by developed countries—in particular, the United States.

Table II-6

Annual Affected Births

WORLD DISTRIBUTION OF HEMOGLOBIN DISORDERS. **DATA COMPILED FOR NIH**
AFFECTED BIRTHS.

AFFECTED BIRTHS PER YEAR: NUMBERS

	TYPE OF SYNDROME												
	CC+C β-Thal	S/C	SS	S/ β-Thal	β-Thal Major	HbE/ β-Thal	HbEE	α-Thal hydrops	HbH	All	Pathol	Percent of all	Percent of pathol
Africa	14,618	46,846	150,848	9,200	4,086	—	—	—	—	225,598	210,980	62.5	71.1
Americas	527	2,126	4,339	952	227	—	—	—	—	8,171	7,644	2.3	2.6
Asia	—	—	20,159	1,963	20,508	15,817	49,289	4,507	13,186	125,429	76,140	34.7	25.7
Europe	—	68	235	89	1,385	—	—	—	—	1,777	1,777	0.5	0.6
Oceana	—	—	—	—	100	—	—	—	—	100	100	0.0	0.0
WORLD	**15,145**	**49,040**	**175,581**	**12,204**	**26,306**	**15,817**	**49,289**	**4,507**	**13,186**	**361,075**	**296,641**	**100**	**100**

Comment: There are almost 300,000 affected births per year. More than 60 percent of the total and 70 percent of the pathological are in Africa.

PROPORTIONS OF THE MAIN GROUPS OF SYNDROMES

	ALL SYNDROMES					PATHOLOGICAL SYNDROMES				
	Total	Sickling disorders	Thalassemias	SCD percent of all	Thals percent of all	Total	Pathol SCD	Pathol Thals	SCD percent of pathol	Thals percent of pathol
Africa	225,598	221,512	4,086	98.2	1.8	210,980	206,894	4,086	98	2
Americas	8,171	7,944	227	97.2	2.8	7,644	7,417	227	97	3
Asia	125,429	22,122	103,307	17.6	82.4	76,140	22,122	54,018	29	71
Europe	1,777	392	1,385	22.1	77.9	1,777	392	1,385	22	78
Oceana	100	—	100	—	100.0	100	—	100	—	100
WORLD	**361,075**	**251,970**	**109,105**	**69.8**	**30.2**	**296,641**	**236,825**	**59,816**	**80**	**20**

Comment: Thalassemias contribute 30 percent of syndromes and 20 percent of pathological syndromes.

TABLE II-6 (continued)

SYNDROME MIX BY REGION

	PERCENT OF ANNUAL HOMOZYGOTE BIRTHS								
	CC+C β-Thal	S/C	SS	S/ β-Thal	β-Thal Major	HbE/ β-Thal	HbEE	α-Thal hydrops	HbH
Africa	6.5	20.8	66.9	4.1	1.8	—	—	—	—
Americas	6.4	26.0	53.1	11.7	2.8	—	—	—	—
Asia	—	—	16.1	1.6	16.4	12.6	39.3	3.6	10.5
Europe	—	3.8	13.2	5.0	77.9	—	—	—	—
Oceana	—	—	—	—	100	—	—	—	—
WORLD PERCENT OF ALL	**4.2**	**13.6**	**48.6**	**3.4**	**7.3**	**4.4**	**13.7**	**1.2**	**3.7**
WORLD PERCENT OF PATHOL.	**—**	**16.5**	**59.2**	**4.1**	**8.9**	**5.3**	**—**	**1.5**	**4.4**

AFFECTED BIRTHS PER THOUSANDS

		ALL SYNDROMES					PATHOLOGICAL SYNDROMES				
	Births/yr (thousands)	Sickling disorders	Thals	SCD per 1,000	Thals per 1,000	Total per 1,000	SCD	Thals	SCD per 1,000	Thals per 1,000	Total per 1,000
Africa	29,786	221,512	4,086	7.44	0.14	7.60	206,894	4,086	6.95	0.14	7.08
Americas	17,606	7,944	227	0.45	0.01	0.50	7,417	227	0.42	0.01	0.43
Asia	83,489	22,122	103,307	0.26	1.24	1.50	22,122	54,018	0.26	0.65	0.91
Europe	11,051	392	1,385	0.04	0.13	0.16	392	1,385	0.04	0.13	0.16
Oceana	524	—	100	—	0.19	0.19	—	100	0.00	0.19	0.19
WORLD	**142,456**	**251,970**	**109,105**	**1.77**	**0.77**	**2.74**	**236,825**	**59,816**	**1.66**	**0.42**	**2.08**

Table II-6 (continued)

PROJECTION OF PATIENT NUMBERS WITH BASIC MODERN MANAGEMENT

DATA COMPILED FOR NIH

AFFECTED BIRTHS PER YEAR: NUMBERS

	TYPES OF SYNDROME										
	CC+C β-Thal	S/C	SS	S/ β-Thal	β-Thal Major	HbE/ β-Thal	HbEE	α-Thal hydrops	HbH	Total	Pathol.
WORLD	15,145	49,040	175,581	12,204	26,306	15,817	49,289	4,507	13,186	361,075	296,641
Estimated survival (yr)	70	60	40	50	30	55	70	0	60	—	—
Cumulative no: Millions	1.06	2.94	7.02	0.61	0.79	0.87	3.45	0.00	0.79	17.54	13
Thalassemia	—	—	—	—	0.79	0.87	—	—	0.79	—	2.45
Sickling syndromes	—	2.94	7.02	0.61	—	—	—	—	—	—	10.58

Chapter III

From Basic Research to Clinical Applications

A. CLINICAL MANAGEMENT

The lives of patients with Cooley's anemia have been dramatically improved by the combination of modern blood transfusion and iron-chelation therapy (figures III-1 and III-2). In 1925, when Dr. Thomas Cooley first described the condition now known as thalassemia major, most children died of anemia by age 2. During the next four decades, red blood cell transfusions were usually given at irregular intervals and only for symptoms of severe anemia such as extreme weakness and fatigue. In addition to severe anemia, patients suffered from stunted growth, cardiac enlargement, retarded maturation and development, and swelling of the abdomen as a result of the enlargement of the liver and spleen. The relentless but futile expansion of the bone marrow in response to severe anemia produced thinning and fractures of long bones and spinal vertebrae along with characteristic distortions of the facial bones and skull (see figure I-2, p. 3). The ineffectual hyperactivity of the marrow was also associated with increased absorption of iron from the gastrointestinal tract and development of iron overload.

Transfusion Therapy

By 1965, clinical research had led to modern programs of regular red blood cell transfusions that transformed the lives of

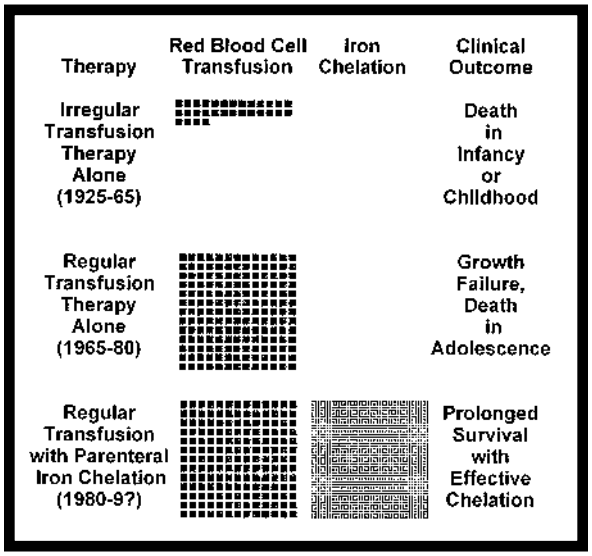

Figure III-1

Evolution of red blood cell transfusion and iron-chelation therapy with deferoxamine for Cooley's anemia. In the central columns, each square represents one unit of packed red blood cells, containing about 250 milligrams of iron; each dot represents one gram (two vials) of deferoxamine. The number of packed red blood cell transfusions illustrated in the lower two rows is the number that a typical patient with Cooley's anemia might have received by age 12 (more than 160 units or about 40 grams of iron). The number of grams of deferoxamine shown in the lowest row is the amount (about 1,600 grams) that would be required for effective chelation therapy of the transfusional iron load shown. The daily dose of deferoxamine is about 50 milligrams per kilogram body weight, or roughly 1.0 gram for patients between ages 4 and 8, 1.5 grams for those between ages 8 and 12, and 2.0 grams for those older than age 12.

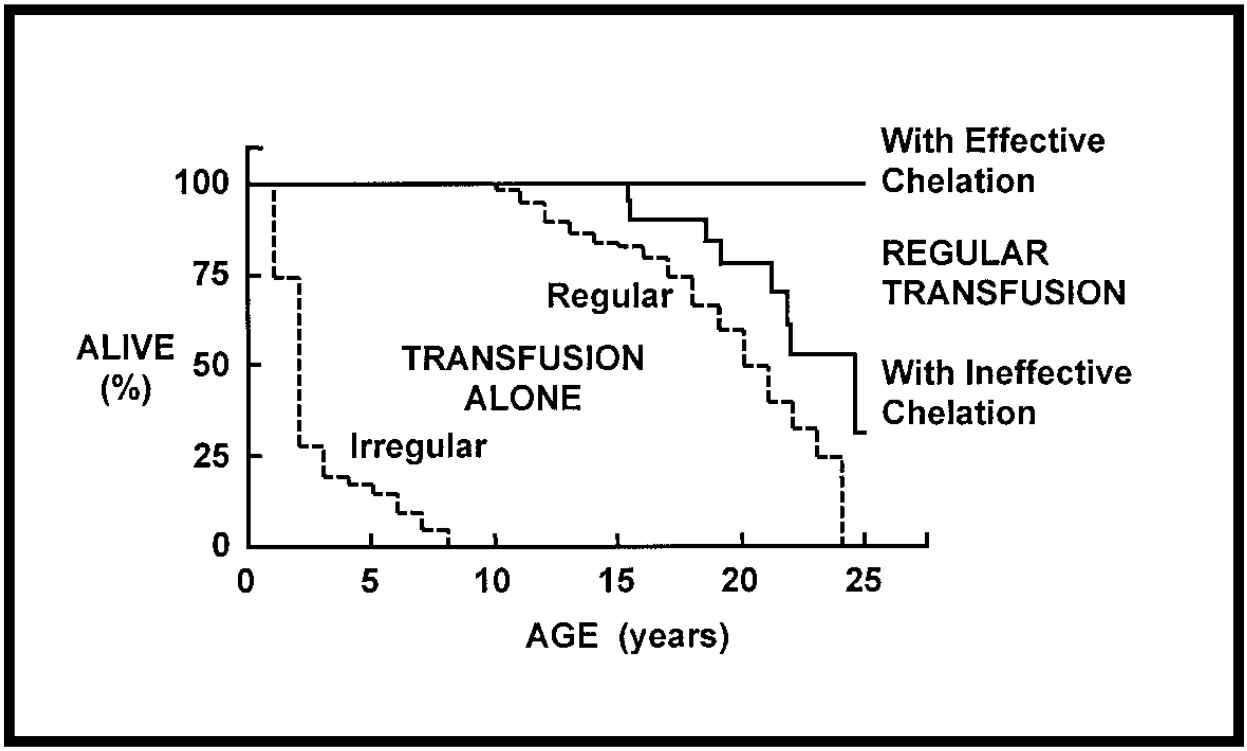

Figure III-2

Representation of the survival of patients with Cooley's anemia with various regimens of red blood cell transfusion and iron-chelation therapy. The broken lines represent the survival of patients treated with red blood cell transfusion alone; the solid lines, the survival of patients treated with red blood cell transfusion and iron-chelation therapy with deferoxamine. [Adapted from: Modell and Berdoukas, The clinical approach to thalassemia. London, Grune & Stratton, 1984; Olivieri, et al., N Engl J Med 1994; 331:574-8; and Brittenham, et al., N Engl J Med 1994; 331:567-73].

patients with thalassemia major. Life-long regimens of regular transfusions begun in infancy could provide continuous treatment for severe anemia, preventing the extreme expansion of the bone marrow with the associated bony distortions and averting enlargement of the liver and spleen.

Research advances in transfusion medicine have led to measures to avoid transfusion reactions by complete genotyping of red blood cells before the first transfusion and by using washed, filtered, or glycerol-frozen red blood cells. Careful screening of blood has minimized the risk of transfusion-transmitted infection. Optimal timing and conditions for removal of the spleen, when necessary, have been determined. Immunization programs with multivalent pneumococcal vaccine and *Hemophilus influenza* type B vaccine before splenectomy, together with prophylactic penicillin after splenectomy, have decreased the risk of infection. The actual risk of postsplenectomy sepsis in this population is unknown, but penicillin therapy is still recommended for patients who have undergone splenectomy, especially during childhood. With red blood cell transfusions every 2 to 4 weeks, patients with Cooley's anemia could now have lives with somewhat normal activity, growth,

and development during childhood. These programs of regular transfusion reduced gastrointestinal iron absorption to near normal amounts, but the iron within transfused red blood cells accumulated steadily, producing a further problem for patients with Cooley's anemia—transfusional iron overload.

Transfusional Iron Overload

Regular transfusion requires substantial amounts of blood. By age 12, a typical patient may have received the equivalent of more than 160 units of red blood cells. Each unit of red blood cells contains a fixed amount of iron as an integral part of the oxygen-carrying hemoglobin. Iron is an essential nutrient required by every human cell; the human body is made to conserve iron and lacks any means to excrete the excess iron that progressively accumulates with regular red blood cell transfusions.

Although the human body has a remarkable capacity to hold the surplus iron, eventually the increased amount overwhelms the ability of the body to safely store the excess. The very reactivity that makes iron so useful as an oxygen carrier in hemoglobin, and as a constituent of vital metabolic compounds, then becomes hazardous. The excess iron may damage a variety of tissues, especially the liver, heart, pancreas, and other endocrine organs. Without specific therapy to remove the iron, by early adolescence almost all regularly transfused patients will have acquired a toxic accumulation of iron. Growth and maturation then slow; liver disease, diabetes mellitus, and other complications develop; and premature death may occur, usually from cardiac involvement (figure III-2).

Iron-Chelation Therapy With Deferoxamine

Treatment with an agent able to bind (chelate) the iron and promote its excretion provides a means to reduce the iron burden in patients dependent upon red blood cell transfusions. In 1962, deferoxamine B mesylate (Desferal®), a naturally occurring iron-chelator produced by bacteria (*Streptomyces pilosus*), was first shown to increase iron excretion in patients with Cooley's anemia. Nearly 20 years of clinical research were then required to learn to use the drug effectively. Deferoxamine is so poorly absorbed after being given by mouth that the drug must be given by injection. In addition, the drug is so rapidly eliminated that prolonged infusions are needed to produce a therapeutic effect. As a result, to be optimally useful in the treatment of patients with transfusional iron overload, deferoxamine must be given by slow subcutaneous or intravenous infusion over 9 to 12 hours each day, a difficult and demanding regimen. At this time, deferoxamine remains the only iron-chelating agent available for clinical use in the United States. By 1980, research in patients with Cooley's anemia had led to the design of treatment programs with daily, prolonged infusions of deferoxamine through portable subcutaneous pumps that could successfully prevent or treat transfusional iron overload. Since then a series of clinical trials with deferoxamine have demonstrated the benefits of iron-chelation in Cooley's anemia patients. Accurate determination of the extent of body iron loading has been essential to guide iron-chelation therapy and to monitor its progress in removing iron. Chemical assay of iron in liver tissue

obtained by biopsy is useful for this purpose, but noninvasive measurements of hepatic magnetic susceptibility now provide quantitatively equivalent results.

Successes of Chelation Therapy

Regular, sustained chelator treatment can lower the body iron burden; decrease liver iron; improve cardiac, pancreatic, and other organ function; improve growth and sexual maturation; and increase survival (figure III-2). Two recent prospective trials have shown that patients able to use amounts of deferoxamine adequate to control the body iron load have a more than 90 percent likelihood of survival to age 25 (figure III-2). By contrast, patients unable or unwilling to use sufficient amounts of deferoxamine for effective chelation had a high risk of complications of iron overload with a less than 30 percent probability of survival to age 25. Both trials identified the magnitude of the body iron burden as a critical factor for survival free of the complications of iron overload. As survival has extended to the second and third decades of life, sexual maturation and fertility have become important issues to be considered in patients with Cooley's anemia. Sustained reduction in body iron burden has been shown to be associated with normal sexual maturation, and many patients with Cooley's anemia have borne or fathered normal children.

Difficulties of Deferoxamine Therapy

Unfortunately, many patients are unable to use sufficient amounts of deferoxamine to prevent the complications of iron overload. Understandably, the need to administer deferoxamine by placing a needle under the skin each night is difficult for many patients. The majority of patients with Cooley's anemia report a negative impact of this onerous regimen on their quality of life, and some are unable to continue this treatment during late childhood and adolescence.

Although a small group of patients are unable to administer deferoxamine because of the development of allergy or toxicity, allergy associated with deferoxamine is rare. Toxic effects of the drug also occur infrequently and are usually the result of an excess deferoxamine dose in patients with relatively small amounts of body iron. The fundamental problem with deferoxamine is that many patients are unable to use sufficient amounts of a drug that requires that they be tied to an irritating infusion for 10 hours a day. In the United States, the inability of patients with Cooley's anemia to comply with prolonged subcutaneous or intravenous infusions represents the primary obstacle to the reduction of body iron and is the reason some patients still die of the complications of iron overload.

The skin irritation associated with subcutaneous use of deferoxamine has prompted many medical centers to give intravenous deferoxamine therapy through implantable ports, which provide access to a large vein through which deferoxamine is infused. This system of deferoxamine delivery often results in dramatic decreases in body iron over a few months, especially if deferoxamine can be infused 24 hours a day through the port. This is, therefore, useful in patients with very high body iron burdens who may be at risk of complications of iron overload. Intravenous administration of deferoxamine may be associated with improved compliance in patients who have been poorly compliant with subcutaneous deferoxamine. This system of deferoxamine administration also has its disadvantages,

when compared with subcutaneous deferoxamine infusions, for example, a higher risk of bacterial sepsis.

General Recommendations for Followup of Chelation Therapy

The regular assessment of chelation therapy in patients with Cooley's anemia should include the monitoring of effectiveness, toxicity, and compliance with deferoxamine. Table III-1 summarizes the deferoxamine-related toxicities. Regular, accurate determination of body iron loading is essential to guide chelation therapy and monitor its progress in the removal of iron. The assessment of liver iron using tissue from liver biopsy or noninvasive measurements of hepatic magnetic susceptibility using the SQUID (superconducting quantum interference device), which provides quantitatively equivalent results, remains the best methods for the determination of body iron loading. The serum ferritin concentration is frequently used to estimate the body iron burden, but this measurement provides only a general guide to the severity of body iron loading. More useful in long-term followup are tests of organ function, including functional assessments of the heart, the pancreas, and the anterior pituitary gland—organs that are most often affected by iron loading. The toxicity of deferoxamine therapy is usually manifested as vision or hearing problems or as abnormalities of growth and bone formation in childhood. Careful examination of these systems should be obtained on an annual basis (table III-2).

Development of Orally Active Iron-Chelating Agents

Despite the proven efficacy of deferoxamine, this chelator is toxic in some patients; is expensive (therapy may cost $10,000 per year or more); and is difficult for patients to use because of the requirement for prolonged, daily subcutaneous or intravenous administration. These limitations have mandated further research to identify safe, inexpensive, and orally active iron-chelating agents as successors to deferoxamine.

Table III-1

Monitoring of Deferoxamine-Related Toxicity

Toxicity	Investigations	Frequency	Alteration In Therapy+
High-frequency sensorineural hearing loss	Audiogram	Yearly; if patient symptomatic, immediate reassessment	Reduce deferoxamine to 25 to 30 mg/kg/day; assess body iron load; repeat audiogram after 3 months
Retinal abnormalities	Full retinal examination	Yearly; if patient symptomatic, immediate reassessment	Discontinue deferoxamine; reassess after 3 months
Metaphysical and spinal abnormalities	X-rays of wrists, knees, spine; bone age of wrist	Yearly	Reduce deferoxamine to 25 to 30 mg/kg/day; reassess after 6 months
Decline in height velocity	As above; determination of sitting/standing height	Yearly	As above; regular (6 month) assessment of pediatric endocrinologist

+The accurate assessment of body iron stores should be undertaken regularly, especially in patients with suspected deferoxamine-related toxicity

Table III-2

Evaluation of the Patient With Thalassemia

INITIAL

- Clinical assessment
- Hematologic evaluation (CBC, Hb electrophoresis)
- α/non-α globin chain synthesis ratio
- Red blood cell phenotype
- Serum iron, total iron-binding capacity
- Serum ferritin
- RBC and serum folates
- Total and direct bilirubin
- Serum ALT, albumin, prothrombin time, and PTT
- Hepatitis screen
- Initiation of hepatitis B vaccine series
- Parental counseling and education

MONTHLY

- History and physical examination
- CBC
- Parental counseling
- Compliance review (if receiving iron chelation therapy)

Q3 MONTHLY

- Serum ALT and ferritin

YEARLY

Age <10 years

Liver	■ Biopsy ■ Hepatitis screen ■ Serum ALT, albumin, PT
Endocrine	■ Serum Ca^{++}, PO_4, Mg^{++}, Zn^{++}, PTH and TSH

Monitoring of deferoxamine toxicity (table III-1)

Age >10 years

Liver	■ Biopsy ■ MRI ■ Hepatitis screen ■ Serum ALT, albumin, PT
Heart	■ Cardiology consultation ■ Radionuclide angiography ■ 24-hour Holter monitoring ■ MRI
Endocrine	■ Endocrinology consultation ■ Fasting blood glucose ■ Serum Ca^{++}, PO_4, Mg^{++}, Zn^{++} ■ Serum PTH and TSH ■ GnRH stimulation test ■ MRI of anterior pituitary

Monitoring of deferoxamine toxicity (table III-1)

A variety of compounds have been produced and examined as potential oral iron-chelating agents, but only one of these is being evaluated in clinical trials: deferiprone (1,2-dimethyl-3-hydroxypyridin-4-one, also known as L1, CP20, and DMHP). Deferiprone was first developed as an iron chelator in 1982 and has undergone preliminary study in the United Kingdom, Canada, Europe, and India. A recent prospective evaluation of this orally active chelator has provided evidence that deferiprone induces sustained decreases in body iron to concentrations compatible with

the avoidance of the complications of iron overload (figure III-3). Unfortunately, some patients have developed severe neutropenia or agranulocytosis during deferiprone therapy. Until the risk of agranulocytosis is determined, deferiprone must be considered an investigational drug restricted to patients unable or unwilling to use deferoxamine. Whatever the ultimate clinical usefulness of this particular chelator, the experience with deferiprone has unequivocally demonstrated the feasibility of effective iron-chelation therapy with orally active agents. This accomplishment has already served to stimulate the search for still safer and more effective iron-chelators that will remain active when given by mouth.

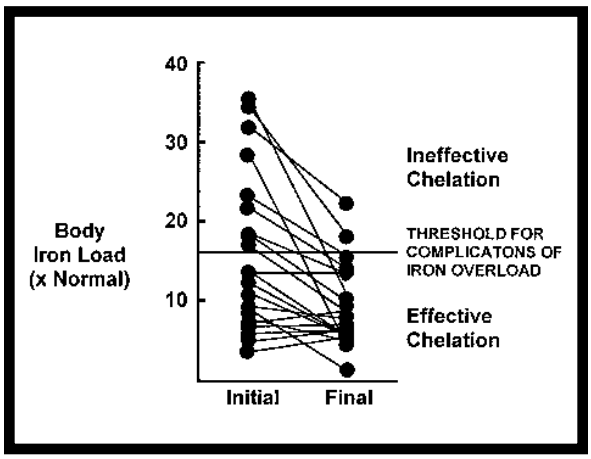

Figure III-3

Estimated body iron burden in 21 patients with thalassemia major treated with the oral iron-chelating agent deferiprone for about 3 years. Estimates of initial and final body iron burden are shown, based on measurements of the hepatic storage iron concentration. The horizontal solid line indicates the level of body iron below which patients treated with deferoxamine remained free of the complications of iron overload. [Adapted from Olivieri, et al., N Engl J Med 1995; 332:918-22].

B. PATHOGENESIS: FROM PROTEIN TO MOLECULAR MECHANISMS

The hallmark of all thalassemias, including Cooley's anemia, is unbalanced synthesis of either the alpha- (α) or the beta- (β) globin chain of hemoglobin. Each chain is produced by the action of a specific gene. Normally, during the formation of red blood cells, the globin chains are produced in a balanced manner that permits the appropriate assembly of hemoglobin. This balanced synthesis is essential if hemoglobin is to perform its vital function of carrying oxygen within the red blood cells to all of the other cells of the body. Unbalanced synthesis, characteristic of the thalassemias, results in the formation of insoluble aggregates composed of the chain made in relative excess. These aggregates interfere with red blood cell production, function, and survival.

The composition of hemoglobin switches twice during life—first during the transition from embryonic to fetal hemoglobin and then, at birth, from the fetal to the "adult" form (figure III-4 B). Embryonic hemoglobin is made up of zeta-(ζ) globin and epsilon-(ε) globin chains. During the last two trimesters of pregnancy, fetal hemoglobin, which is composed of two alpha-(α) globin chains and two gamma-(γ) globin chains, predominates. Fetal hemoglobin has a particularly high affinity for oxygen, enhancing the ability of the fetus to obtain sufficient oxygen from the mother's blood across the placenta. At birth, the genes that produce the fetal or gamma-globin chains switch off and the beta-globin genes are turned on. The switch results in adult hemoglobin, each molecule of which is made up of two alpha-globin chains and two beta-globin chains.

Genetic mutations can disrupt the balanced synthesis of the globin chains. The human alpha-globin gene cluster is located on chromosome 16 and the beta-globin gene cluster on chromosome 11 (figure III-4 A). Deletions or mutations in these clusters affect the gene's ability to produce the globin chains needed to assemble functional adult hemoglobin. Depending on the nature of the mutation, globin synthesis may be affected minimally or markedly, leading to mild or severe forms of thalassemia.

Cooley's anemia, the most severe form of beta-thalassemia, occurs when both beta-globin genes are defective (that is, the individual is homozygous for the genetic error). Most often, the defect arises from a point mutation, wherein a single nucleotide in the DNA sequence is substituted for the correct nucleotide. Some patients inherit a chromosome that is missing the entire beta-globin gene. Both of these genetic scenarios usually result in severe anemia and the need for regular blood transfusions. There are two possible outcomes if only one of the two beta-globin genes is defective (heterozygosity). A small percentage of these individuals, known as "silent carriers," have normal red blood cells and no symptoms. More frequent are carriers who have unusually small red blood cells and a very mild anemia.

Modern molecular biology has identified more than 200 mutations that can occur in individuals with thalassemia. Specific mutations have been shown to affect each step in the flow of genetic information that leads to the production of beta-globin chains. This process is illustrated in figure III-5. Detailed study of these mutations has provided important information about the precise nucleotide sequences required for each step in expression of the beta-globin gene. Of particular importance is the finding that specific genetic mutations can be correlated with disease severity. For example, certain point mutations reduce transcription of the beta-globin gene only moderately. People who are homozygous for these mutations are diagnosed with "β^+ thalassemia" and may not require blood transfusions. In contrast, people who are homozygous for a point mutation that prematurely terminates protein synthesis have red blood cells that lack beta-globin entirely. In these cases (termed β°-thalassemia), anemia is severe and blood transfusions are required.

Deletions of alpha-globin genes are very common in certain areas of the world. Some patients with Cooley's anemia also inherit a mutation that decreases the synthesis of alpha-globin chains. When this happens, the disease takes a milder form. Researchers have shown that the decreased synthesis of alpha-globin chains reduces the imbalance of the globin chains and lessens the formation of insoluble alpha-globin chain aggregates. These findings provided the first clear molecular evidence of a "genetic modifier" that interacts with disease-causing mutations to influence the clinical severity of the disease.

In this regard, the systematic study of human hemoglobins during the past three decades has identified a condition termed "hereditary persistence of fetal hemoglobin" (HPFH), in which the blood contains unusually high levels of fetal hemoglobin. Normally, as a result of the "switch" to adult hemoglobin, the blood contains less than 1 percent fetal hemoglobin. In contrast, individuals with HPFH can have from 20 percent to 100 percent fetal hemoglobin in their blood.

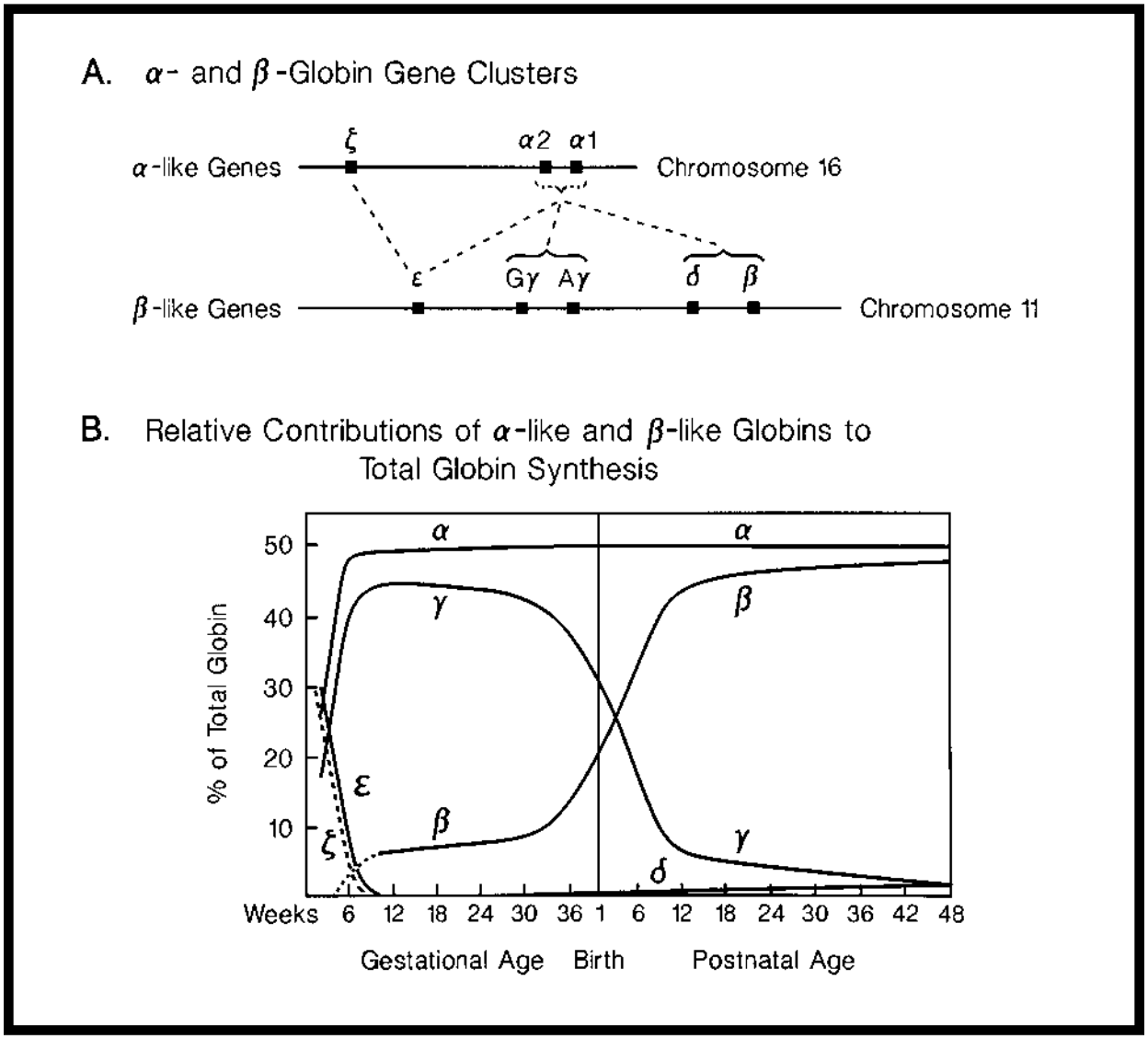

Figure III-4 A

The globin gene loci. The alpha-gene cluster, located on chromosome 16, contains three functional genes, the duplicated alpha-genes (α1 and α2) and the embryonic ζ-globin gene. The beta-like genes are located on chromosome 11. This gene cluster includes two adult globin genes (delta and beta, δ and β), two fetal globin genes (G-gamma and A-gamma, $^{G}\gamma$ and $^{A}\gamma$) and the embryonic epsilon-gene (ε-gene).

Figure III-4 B

Hemoglobin switching. During normal development, there are characteristic changes in the level of expression of the individual globin genes leading to the formation of distinct hemoglobin species. For example, fetal hemoglobin ($Hb\alpha_2\gamma_2$) is replaced at about the time of birth by adult hemoglobin ($Hb\alpha_2\beta_2$). In patients with Cooley's anemia, the defect in hemoglobin synthesis becomes evident at the time of this switch as the defective beta-genes are activated.

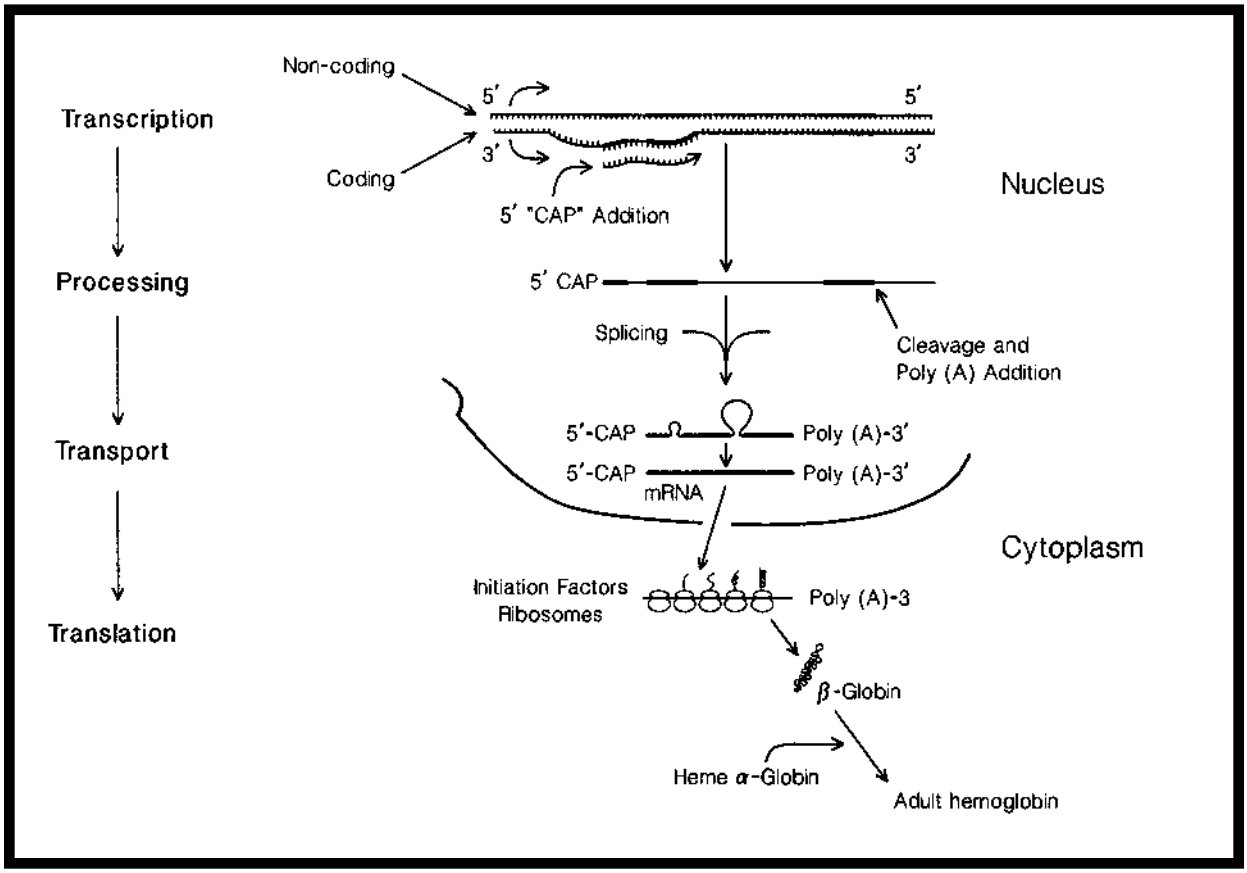

Figure III-5

Expression of globin genes. The flow of genetic information from the DNA sequence to the final protein product involves several discrete steps. First, the coding strand is copied into RNA by a process called transcription. Processing of the RNA species includes modifications of the 5′ end, referred to as capping, addition of adenosines on the 3′ end, and splicing to remove intron sequences. The final mRNA species is transported to the cytoplasm where it is translated into protein. The alpha- and beta-like chains assemble spontaneously to form hemoglobin molecules. Mutations that cause thalassemia may interfere with any one of these major processes, namely transcription, processing, transport, or translation.

The mutations that cause HPFH are important in understanding Cooley's anemia because they also have the potential to act as genetic modifiers. HPFH mutations linked to the gamma-globin genes or located on another chromosome can affect the potential for synthesis of fetal hemoglobin in adults. Coinheritance of these mutations in patients with Cooley's anemia can reduce the severity of the disease. For example, individuals who coinherit a point mutation that causes a significant increase in fetal hemoglobin, along with a mutation in each of the beta-globin genes that decreases beta-globin synthesis, may have a very mild thalassemia syndrome.

The entire beta-globin gene cluster, which includes the fetal gamma-gene and the adult beta-gene, is influenced by a segment of DNA called the LCR, for locus control region. The LCR controls activity of the region of chromosome 11 containing the beta-gene cluster. The LCR permits expres-

sion of specific globin genes during various developmental stages and influences the level to which these genes are expressed. Molecular analysis suggests that the gamma- and beta-globin genes within the beta-globin gene cluster compete for activating sequences in the LCR. Studies designed to clarify the elements involved in this competition may prove relevant to the treatment of Cooley's anemia with gene therapy, as discussed in a later section.

The findings summarized here represent a triumph of molecular medicine and have led to a rich and detailed understanding of the molecular basis of the thalassemias. The genetic principles that have emerged from this research—particularly the demonstration that both the exact nature of mutations and coinheritance of genetic modifier genes determine disease severity—provide models for future studies of thalassemias, inherited hemoglobinopathies in general, and other genetic disorders. Further, these studies provide the foundation for development of gene therapy approaches to Cooley's anemia.

C. FETAL HEMOGLOBIN AND PHARMACOLOGIC MANIPULATION

As introduced in the previous section, prior to birth, beta-thalassemia fetuses develop normally because they make an alternate hemoglobin called fetal hemoglobin in which two alpha-globin chains are combined with two gamma-globin chains ($\alpha 2\ \gamma 2$). A reduction in gamma-globin production and compensatory increase in beta-globin production (the hemoglobin "switch") occurs during the third trimester, shortly before birth. This results in the production of adult hemoglobin ($\alpha 2\ \beta 2$). In patients with beta-thalassemia, the defective beta-globin gene cannot be fully switched on and the newborn infant becomes very anemic. If the hemoglobin switch can be delayed or if fetal hemoglobin production can be turned back on (reverse of the normal switch from fetal to adult hemoglobin), then the anemia caused by the defective beta-thalassemia genes can be reduced or corrected.

It has long been a goal of scientists to understand how the switch from gamma- to beta-globin production works and to possibly stop this switch or reverse it (figure III-6). It had been well known that certain genetic mutations around the gamma-globin genes result in elevated fetal hemoglobin production throughout adult life. These HPFH mutations were discussed in the previous section. Also, under conditions of erythroid stress, such as recovery from severe anemia or marrow failure, fetal hemoglobin transiently increases. This capacity to increase fetal hemoglobin production during adult life had been shown to be a common feature of all normal individuals when red blood cells were grown in erythroid cultures where very high levels of fetal hemoglobin could be produced. It was not, however, until animals treated with an anticancer drug, 5-azacytidine, that it became apparent that pharmacologic agents could actually induce fetal hemoglobin production. Shortly after this observation, it was shown that 5-azacytidine could increase fetal hemoglobin production in patients with thalassemia major and sickle cell disease.

Since the initial observation that 5-azacytidine could increase gamma-globin production, three different classes of pharmacologic agents have been shown to increase fetal hemoglobin levels in patients with beta-thalassemia. The first group of agents are drugs that affect the way that cells divide and/or inhibit DNA synthesis. These cytotoxic agents had heretofore been used

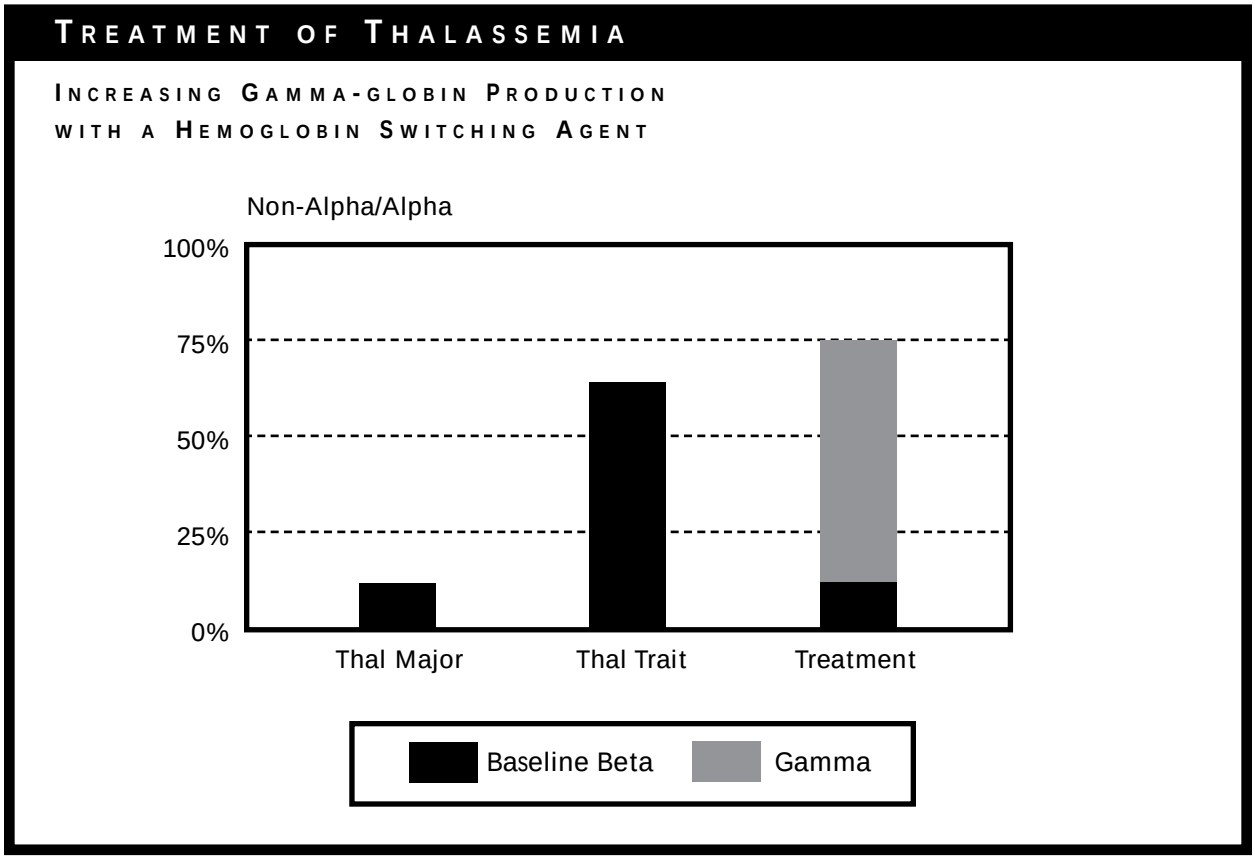

Figure III-6
Increasing gamma-globin production with a hemoglobin switching agent.

as anticancer drugs, but, in lower doses, all had been shown to increase fetal hemoglobin in patients with beta-thalassemia. A second class of pharmacologic agents that induce fetal hemoglobin production are hematopoietic growth factors. These naturally occurring substances, such as erythropoietin, modulate the production of blood cells. Possibly by recreating the effects of erythroid stress, erythropoietin at very high doses also transiently increases fetal hemoglobin production in patients with thalassemia (table III-3). Finally, it was shown that a fatty acid, arginine butyrate, could induce significant levels of fetal hemoglobin production in several thalassemia patients. This led to observations that a variety of fatty acids appear to induce fetal hemoglobin production *in vitro.*

Several clinical trials using single agents or combinations of these agents have begun in the last decade and continue. Both 5-azacytidine and hydroxyurea have been shown to increase gamma-globin production in thalassemia patients. However 5-azacytidine, to date, appears to reduce anemia more substantially than hydroxyurea. Marginal reduction in anemia has been obtained with very high doses of erythropoietin. The degree to which anemia is reduced with these two classifications of agents has been variable, but, for the most part, transfusion-dependent thalassemia major patients have not

Table III-3

Pharmacologic Agents That Induce Fetal Hemoglobin Production

I. Cytotoxic Agents
   5-azacytidine
   hydroxyurea
   (Cytosine arabinoside, vincristine, myleran)*

II. Recombinant Hematopoietic Growth Factors
   Erythropoietin
   (IL-3, GMCSF)*

III. Fatty Acids Analogs
   Arginine Butyrate
   Sodium Phenylbutyrate
   (Isobutyramide, Valproic Acid, Propionic Acid)*

*Drugs in parentheses have been shown to be active in animals or in tissue culture.

uniformly responded sufficiently to these two classes of drugs to become transfusion independent.

An increase in gamma-globin production and actual balance of α /non α -globin chain ratios was seen in one patient treated with intravenous arginine butyrate. This patient had a rare form of beta-thalassemia (homozygous hemoglobin Lepore). However, larger clinical trials with arginine butyrate have not shown such dramatic responses in the more common forms of beta-thalassemia. An oral dosage form of butyrate, sodium phenylbutyrate, previously used to treat congenital metabolic disorders, has also been shown to increase fetal hemoglobin. However, like arginine butyrate, sodium phenylbutyrate has resulted in only minimal-to-moderate increases in total hemoglobin, primarily in patients with thalassemia intermedia and not in patients with transfusion-dependent thalassemia major.

Clinical trials continue to determine whether new agents, such as other fatty acid analogs, can increase in fetal hemo-globin, and more importantly, reduce anemia. In addition, *in vitro* data suggest that all three classes of drugs (cytotoxic drugs, hematopoietic growth factors, and fatty acids) may be additive or synergistic and, therefore, limited trials using combinations of these agents have been started. A multicenter phase III trial of hydroxyurea in sickle cell disease has shown that fetal hemoglobin can be increased sufficiently to reduce the clinical symptoms in some patients with sickle cell disease (decreased vaso-occlusive crises and pulmonary chest syndromes). However, relatively smaller increases in fetal hemoglobin result in clinical benefit in sickle cell disease patients because the fetal hemoglobin inhibits sickle hemoglobin polymerization (figure III-7). Thalassemia patients would require larger increases in fetal hemoglobin to provide a substantial rise in total hemoglobin.

In summary, progress in our ability to reverse the hemoglobin switch and turn on fetal hemoglobin has increased in the past decade, giving promise that a new, effective form of therapy for thalassemic patients may soon be available. However, further research is necessary to understand more precisely how these agents induce fetal hemoglobin production, and additional clinical trials are needed to test the effectiveness and safety of these new switching agents as they are identified.

D. STEM CELL AND BONE MARROW TRANSPLANTATION

Hematopoietic Stem Cell Biology

Blood cells develop from immature progenitors called stem cells. Although very rare in relation to other blood cells, most stem cells are found in the bone marrow of the hips, back, chest, skull, legs, and arms

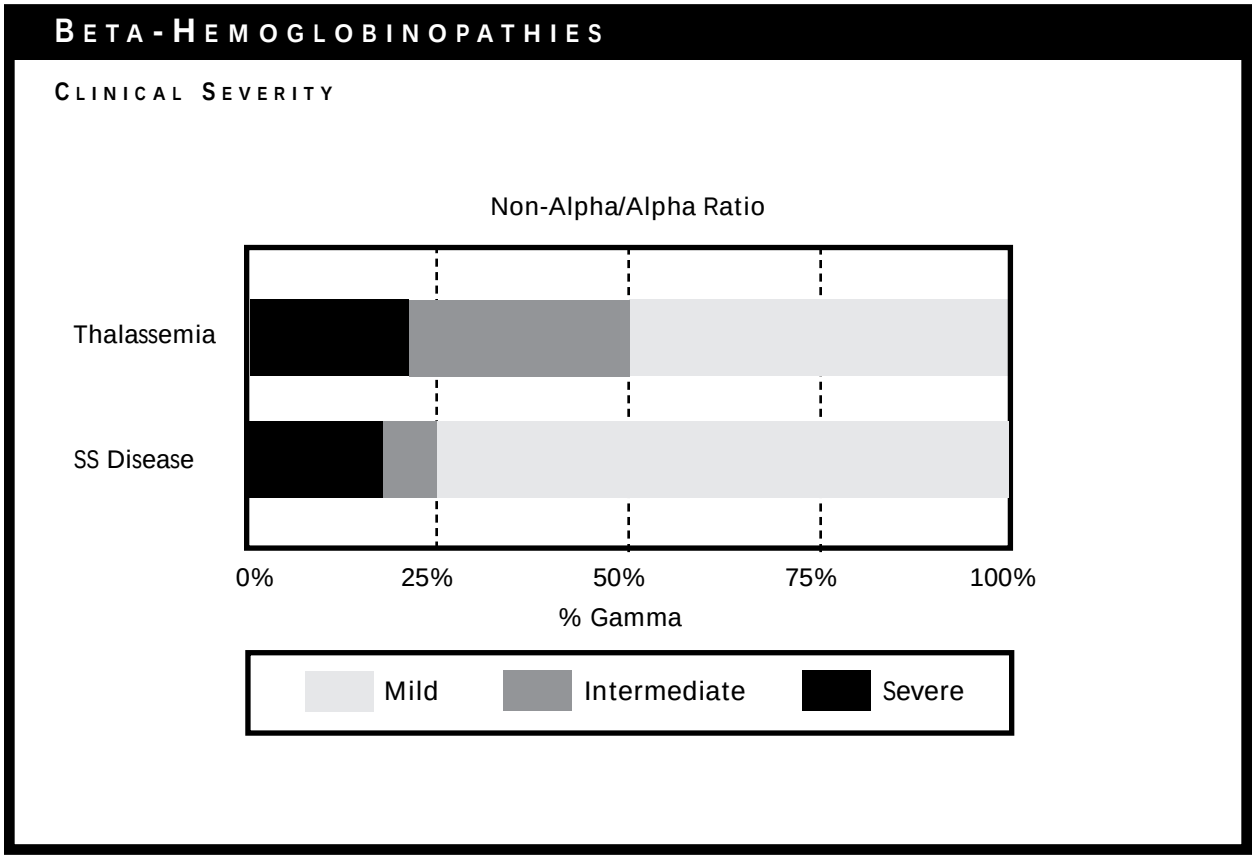

Figure III-7
Clinical severity.

where they make up about 1 out of every 10,000 nucleated marrow cells. Even rarer are peripheral blood stem cells that circulate in the blood throughout the vasculature. The production and differentiation of blood cells is called hematopoiesis. As shown in figure III-8, pluripotent stem cells are the primordial undifferentiated blood cells and can divide to form additional stem cells or can differentiate in the marrow to form more mature blood elements that include red cells, white cells, and platelets. Life-threatening situations may develop if the production of one or more of the blood cell types diminishes.

In the absence of effective production, red blood cells and platelets can be replaced by component-specific blood transfusions, although the benefit from single transfusions is brief.

Hematopoietic growth factors are now available that can stimulate development of cells in the bone marrow. Recent research advances have allowed expansion and mobilization by growth factors and isolation of the elusive human hematopoietic stem cell. Stem cells have been isolated using several methods, including separation of cells via surface markers and high-speed laser sorting. Hematopoietic stem cells can now be isolated and expanded in tissue culture with reliability and purity, making stem cell transplants possible. Alternatively, removal of contaminating lymphocytes without reduction in engraftment potential would enhance the safety of stem cell

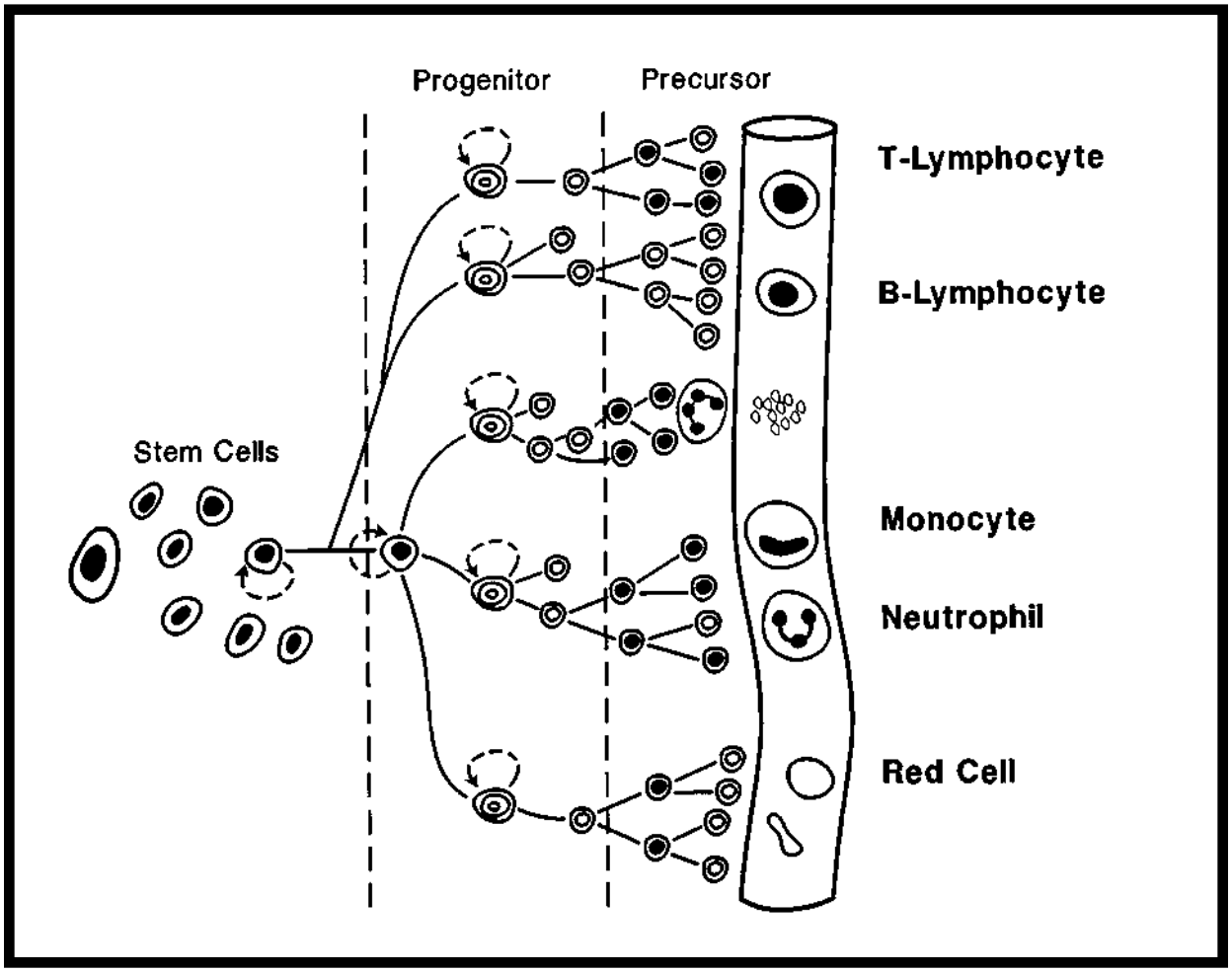

Figure III-8
Outline of normal hematopoiesis (see page 42 for more detailed description of this figure).

transplants from mismatched or unrelated individuals. The following summarizes current applications of stem cell transplantation and future directions in this exciting and fundamental area of research.

Clinical Development of Hematopoietic Stem Cell Transplantation

Since its introduction into clinical medicine more than two decades ago, bone marrow transplantation has achieved increasing success in the cure of otherwise lethal hematologic malignancies, hematologic and immunologic disorders, and certain inborn errors of metabolism. Initial transplant studies were limited to patients with immediately life-threatening hematologic disorders such as aplastic anemia (severe bone marrow failure) and refractory acute leukemia. This pivotal initial experience demonstrated that a proportion of patients (about 50 percent of patients with aplastic anemia and 15 percent of patients with advanced leukemia) could be cured of an otherwise lethal disorder after marrow

transplantation from a sibling donor. Progress over the past decade has been remarkable. Recent reports indicate a 92 percent long-term survival for patients with aplastic anemia and an 81 percent 5-year survival for patients with early stage chronic myelogenous leukemia. Improved supportive care techniques, molecular determination of donor matching, and prevention of adverse graft-host interactions have broadened the application of the procedure to older patients and to those with mismatched or unrelated donors.

Source of Hematopoietic Cells for Transplantation

The source of stem cells for transplantation depends upon both availability of a suitable donor and the underlying disease of the patient. In autologous transplantation (i.e., stem cells from oneself), the patient's own marrow or peripheral blood stem cells are harvested and, in patients with malignancies, reinfused as a stem cell rescue after high-dose chemotherapy or total body irradiation has been given as anticancer treatment. At present, this approach would be of little value in the treatment of Cooley's anemia because the autologous stem cells also carry the thalassemia defect. However, future research advances may allow for transplantation of genetically corrected autologous stem cells.

Allogeneic transplantation (i.e., stem cells from another person) for Cooley's anemia uses a sibling or closely matched related donor who is free of thalassemia major. More recently, unrelated volunteer donors have been identified through marrow registries such as the 1.6 million Americans participating in the federally funded National Marrow Donor Program (NMDP). Success of these unrelated transplants depends upon the degree of genetic similarity between the donor and recipient for HLA present on the surface of white blood cells. Increasing degrees of mismatch (HLA disparity) between the donor (graft) and the patient (host), increase the likelihood of either rejection of the donor graft or an immunologic reaction of donor lymphocytes against host tissues. In other words, the donor's (graft) immunological system begins to reject the host or patient (graft-versus-host disease, GVHD).

In the United States, there is roughly a 30 to 40 percent chance of an individual having an HLA-identical sibling or other closely matched family member. Thus, the majority of patients do not have a matched related marrow or blood stem cell donor. Among Caucasians, the probability of finding an unrelated donor in the NMDP approaches 50 percent, but the odds of finding a donor match are much reduced among minority ethnic groups. Because of the increased risk of GVHD with unrelated donors, matched family donors (if available) are the preferred source of allogeneic stem cells for transplantation.

When donor bone marrow is used, it is harvested by multiple needle punctures and aspirates of the pelvic bones. Donors have 1 to 4 pints of marrow collected in the operating room under general anesthesia and are generally discharged from the hospital the same day. More recently, it has been shown that peripheral blood stem cells may also be used. In this procedure, the donor's blood is circulated through a leukapheresis machine. Over the course of several hours, the stem cells are removed and the donor's blood is returned to the donor. It has become standard to administer

hematopoietic growth factors to the donor for several days before and during leukapheresis procedure as a means of releasing or mobilizing the stem cells from the donor's marrow and thus enhancing the collection of circulating stem cells.

Patients require pretransplant preparative conditioning to (1) destroy the host's immune cells, which are capable of mediating graft rejection and (2) destroy the patient's bone marrow cells as a means of creating space for the new graft and to eliminate the cells that carry the disease. Damage to normal cells is an unavoidable side effect of this chemotherapy. Nausea, oral discomfort, and low blood counts usually resolve within several weeks. After completion of pretransplant conditioning, the marrow or peripheral blood stem cells are infused into the patient, circulate through the bloodstream, and "home" to the bone marrow cavities. Within 2 to 4 weeks, blood counts increase as the graft becomes functional.

Hematopoietic Stem Cell Transplantation for Cooley's Anemia

The first marrow transplant for thalassemia was performed in Seattle in 1981, and the patient has remained well and free of Cooley's anemia. Subsequent to this report, a group in Pesaro, Italy, has carried out more than 700 transplants for thalassemia. Most patients have been children younger than age 16 with HLA-identical sibling marrow donors. Figure III-9 shows the results for 491 patients transplanted between 1981 and 1992. The overall survival rate was about 82 percent and event-free survival was about 75 percent (an event being defined as graft rejection, recurrence of thalassemia, or death). The Pesaro group has further reported outcome according to the presence or absence of pretransplant liver enlargement or liver fibrosis. As shown in figure III-10, Class I patients, those without liver enlargement or liver fibrosis, had long-term survival of 94 percent. This experience has been updated and confirms a 90 percent event-free survival in 111 such Class I patients (table III-4). Note that Class I patients represent only approximately 20 percent of the children with thalassemia. Among Class III children who had a history of irregular chelation, portal fibrosis, and liver enlargement, transplant-related mortality was significantly increased. In a series of young adults with Class II and III disease, event-free survival was 74 percent.

Outside Italy the experience with stem cell transplants for Cooley's anemia is more limited. As of 1994, seven publications, each reporting on 10 or more patients, reported a total of 180 HLA-identical transplant recipients. For the entire cohort, event-free survival was 66 percent and overall survival

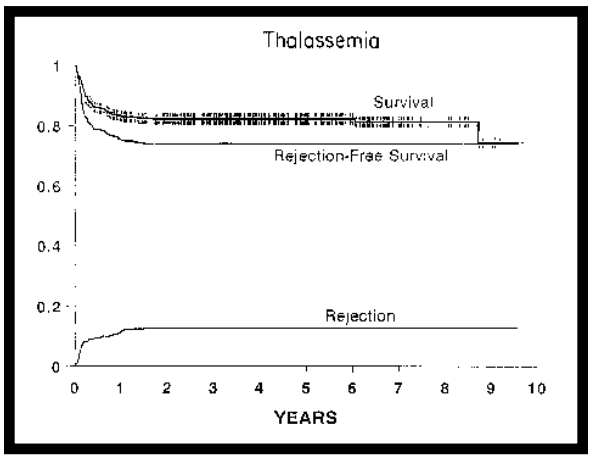

Figure III-9
Probabilities of survival, rejection-free survival, and rejection for 491 patients with Cooley's anemia who received marrow transplants from HLA-identical family members in Pesaro, Italy, between December 1981 and February 1992. Each tic mark represents a surviving patient.

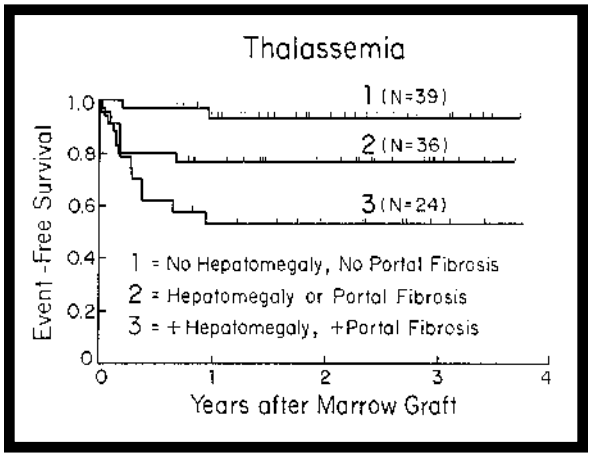

Figure III-10

Probability of event-free survival after marrow transplantation for thalassemia grouped by the presence or absence of hepatomegaly (liver enlargement) and portal fibrosis of the liver. An event was defined as graft rejection, recurrence of thalassemia, or death.

Table III-4

Transplant Outcome in 546 Patients With Cooley's Anemia. Numbers of Patients Are Shown in Parentheses.

MARROW TRANSPLANTATION FOR THALASSEMIA*
(Pesaro, Italy)

Children <17 Years	**TRM**	**Rejection**	**Survival**	**EFS**
Class I (N=111)**	5%	5%	95%	90%
Class II (N=257)**	12%	6%	86%	82%
Class III (N=120)				
BU (14) + Cy (200)	42%	9%	55%	53%
BU (14) + Cy (120)	0	33%		
BU (16) + Cy (120)	13%	28%		
BU (16) + Cy (160)	13%	20%		
Adults (N=58)	22%	4%	78%	74%

*G. Lucarelti, ASH, Dec 93 and NEJM 329:840, 1993
**BU (14 mg/kg) + Cy (200 mg/kg)
TRM, transplant-related mortality
EFS, event-free survival

was 79 percent. In a 1995 report from the United Kingdom, 29 children with thalassemia major were transplanted from HLA-identical family members. Eight were Class I patients, 13 Class II, and 8 were Class III by the Pesaro classification. All 29 children currently survive with event-free survival in 22 (76 percent) patients. With modification of the transplant regimen, there have been no rejections seen in the last 15 patients in this series. Successful transplantation of matched umbilical cord-blood stem cells into a patient with severe thalassemia has been reported by investigators in Bangkok.

The worldwide experience with mismatched or unrelated stem cell transplants for thalassemia is quite limited. Three unrelated and 18 mismatched related transplants have been performed for Class III patients. Of the 21 patients, 5 survive free of thalassemia and 8 survive with return of thalassemia after rejection of the mismatched marrow.

Risks and Complications

On average, patients are discharged from the hospital 15 to 25 days after transplantation. Platelet and red cell transfusions must continue for another 1 to 2 months, and full immune competence requires an additional 6 to 9 months to recover. Accordingly, patients are often advised not to return to school or work for 6 to 9 months after transplantation. In addition, immunosuppressive drugs such as cyclosporine are administered for 6 months after allogeneic transplant to suppress GVHD. Acute GVHD may develop within the first 100 days of transplant and is characterized by skin rash, jaundice, and diarrhea. The risk of developing acute GVHD ranges from 10 to 20 percent in children given matched sibling transplants, to 20 to 40 percent in older recipients of related

grafts, to 80 percent in recipients of mismatched related or unrelated transplants. Chronic GVHD may develop after day 100 and shares features similar to naturally occurring autoimmune diseases. The risk of developing chronic GVHD is lowest (i.e., 10 to 25 percent) in children given HLA-identical donor grafts. Treatment with immunosuppressive drugs is effective in 60 to 85 percent of patients. Less common than GVHD, graft failure may arise either due to failure of the donor stem cells to engraft and produce new blood cells or due to the patient's immune-mediated rejection of the donor graft. In patients with Cooley's anemia, graft failure is evidenced by absence or loss of donor-derived blood cells and the return of thalassemia.

Thus, patients may experience several transplant-related complications. Many are temporary and relatively modest, but others may be severe or life threatening. Advanced patient age, prior medical complications, and increasing donor mismatch increase these risks. Transplant-related mortality ranges from 5 to 10 percent in young patients transplanted from HLA-matched sibling donors, to 15 to 40 percent in older patients or those with mismatched or unrelated donors. The risk of transplant-related mortality has fallen substantially during the past decade due to more effective regimens to prevent infection, GVHD, and rejection. As transplants become safer, this treatment option will become more acceptable for patients with serious but not immediately life-threatening blood diseases such as Cooley's anemia.

Long-Term Followup After Hematopoietic Cell Transplantation

The fate of tissue iron overload has been studied in 151 patients with Cooley's anemia after stem cell transplantation. Serum ferritin and unbound iron-binding capacity returned to within normal ranges only in Class I patients. For Class II and III patients (with the greatest pretransplant iron overload), iron overload persisted for more than 5 years posttransplant. For these patients, a program of iron removal by phlebotomy (removal of blood) or chelation therapy may be required to speed iron removal.

Because long-term pretransplant iron overload in poorly chelated patients may influence reproductive endocrine function, growth, and development, it is difficult to dissociate the effect of the disease and the effect of transplantation on these parameters. Based upon bone marrow transplantation studies of patients with hematologic malignancies given pretransplant busulfan, it is likely that the majority of patients will have gonadal ablation and infertility following this chemotherapy. Although cyclophosphamide has no effect on growth velocity, busulfan causes a temporary delay with subsequent catchup in growth. Chronic GVHD is also associated with late complications. Among patients developing chronic GVHD, 4 to 15 percent of surviving patients develop significant disabling complications.

The importance of measuring quality of life after hematopoietic cell transplantation is well recognized and key to accurately informing patients about the procedure. An operational consensus is emerging among researchers that quality of life is a multidimensional construct minimally composed of four domains: physical function, psychological function, social role function, and disease- or treatment- related symptoms. Several studies of stem cell recipients have found that symptoms of anxiety and fatigue persist for months to years after transplantation. By 3 to 5 years after transplant, concern for disease recurrence or graft rejection

has lessened and the dynamics of recovery become complete. A recent, detailed multidimensional analysis of quality of life in 125 adults more than 5 years after allogeneic transplantation for leukemia found that 80 percent rated their current quality of life as good to excellent, 5 percent rated it as poor, and 88 percent judged that the gains outweighed the risks of the procedure.

Choice of Transplant and Informed Consent

The choice of hematopoietic cell transplantation for the treatment of Cooley's anemia depends on the availability of a suitably matched stem cell donor. For patients unwilling or unable to be treated with chelation and transfusions (especially older children refusing to continue daily deferoxamine infusions), hematopoietic cell transplantation may be the treatment of choice. The projected event-free survival of 53 to 82 percent of patients with stem cell transplant is noteworthy, especially when compared with an untreated natural history of progressive iron overload and death.

Choices are less clear cut for patients fully compliant with long-term chelation and transfusion therapy. Many experts would advise continuing this program for as long as tolerated. Others point to the potential for curative treatment with stem cell transplantation, albeit with clear risk for transplant-related mortality and morbidity. For Class I patients with perhaps a 90 percent chance of event-free survival with transplantation, the weighing of risks versus gains is critical. Because there are no randomized, controlled trials comparing chelation therapy with hematopoietic cell transplantation, the choice must be fully weighed by the patient, parents, and physicians. The choice between these alternative treatments must be based on accurate and detailed information. Medical, financial, social, and psychological factors influencing the decision should be openly discussed. Risks and quality of life with each treatment must be detailed as accurately as possible. When knowledge is incomplete, these limitations and uncertainties should also be presented to aid in informed consent and decisionmaking.

E. GENE THERAPY

Currently, the only curative therapy for Cooley's anemia is bone marrow transplantation. The potential morbidity and possible mortality associated with transplantation and the limited availability of suitable bone marrow and stem cell donors restrict the applicability of this approach. Advances in molecular medicine now suggest that gene therapy holds substantial promise for Cooley's anemia and other hemoglobinopathies.

In principle, gene therapy is straight-forward. Once a specific causative genetic defect is identified, a normal gene is "inserted" into the appropriate target cells to fulfill the functions of the defective gene (figure III-11). The genetic abnormalities that give rise to Cooley's anemia are well known, and the target cells, the hematopoietic stem cells, are accessible in the bone marrow. In patients whose own bone marrow has been destroyed by drugs and replaced with healthy marrow from a genetically matched sibling, normal hemoglobin is synthesized, demonstrating the curability of the disease. The ability to insert a therapeutic gene into stem cells and to achieve a high level of regulated gene expression in the progeny of these multipotential cells (figure III-12) would provide the foundation for curative therapy for the majority of patients with

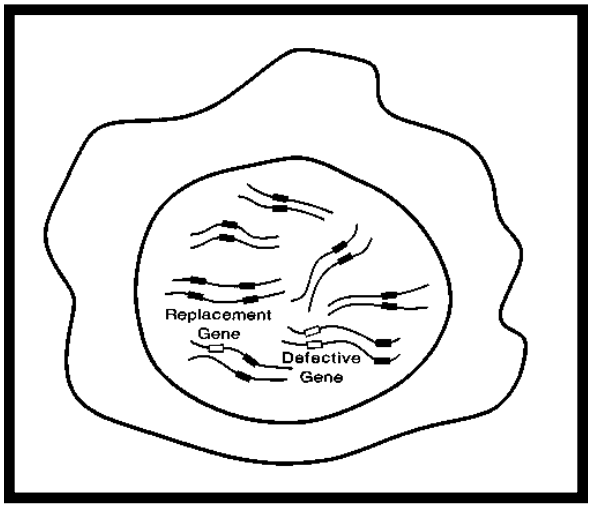

Figure III-11
Gene therapy for genetic disease. All cells contained two copies of most genes with one copy on each of the two homologous, paired chromosomes. Individuals with Cooley's anemia have two defective beta-globin genes. It is the goal of gene therapy to insert a "replacement gene" that restores the missing gene product encoded by the defective genes. As currently envisioned, the replacement gene would be inserted randomly. In the future, it may be possible, through a process of homologous recombination, to actually correct one or both of the defective genes.

Cooley's anemia who are not candidates for bone marrow transplantation. Because the primary defect in Cooley's anemia is decreased production of beta-globin, this disease represents a prototype for gene replacement therapy. Much has been learned about the regulatory elements necessary for high-level globin gene expression. It may ultimately prove feasible to achieve sufficient expression in erythroid cells to functionally correct the deficiency that causes thalassemia. Full correction of the deficiency in beta-globin synthesis is unnecessary, as even heterozygous individuals who have a 50 percent reduction in beta-globin synthesis are entirely asymptomatic.

Yet substantial obstacles must be overcome before this therapy becomes a reality. Research is under way at several institutions in the United States and Europe to overcome these obstacles. As noted above, the "target" cell in devising effective gene therapy for Cooley's anemia is the hematopoietic stem cell. This primitive, self-renewing cell that gives rise, through the process of differentiation, to the erythroid, myeloid, and lymphoid cells in the circulating blood (figure III-12). In spite of advances in the isolation and enrichment of stem cells, large numbers of purified populations of stem cells are still difficult to obtain. To provide adequate numbers of stem cells for use as targets in gene therapy, researchers continue to seek effective techniques to stimulate stem cell division in culture *(ex vivo)* without compromising these cells' ability to self-renew and to produce blood cells of multiple lineages. Maintaining these multipotential properties is a key to subsequent long-term expression of the therapeutic gene in the body. To do this, it will be necessary to overcome the tendency of stem cells to differentiate in culture and, thereby, to lose their multipotential and long-term repopulating capabilities. Although much progress has been made, it is not yet possible to transfer an intact globin gene, along with the regulatory elements necessary to achieve very high levels of globin gene expression in differentiating human erythroid cells.

The majority of gene therapy research has been based on the use of modified retroviruses to transfer the therapeutic gene into the target cells. A frequently used retroviral vector is a murine leukemia virus. The retrovirus is structurally altered to ensure that it cannot replicate (and hence cannot cause infection) and is engineered to carry only the gene of interest, the DNA sequences

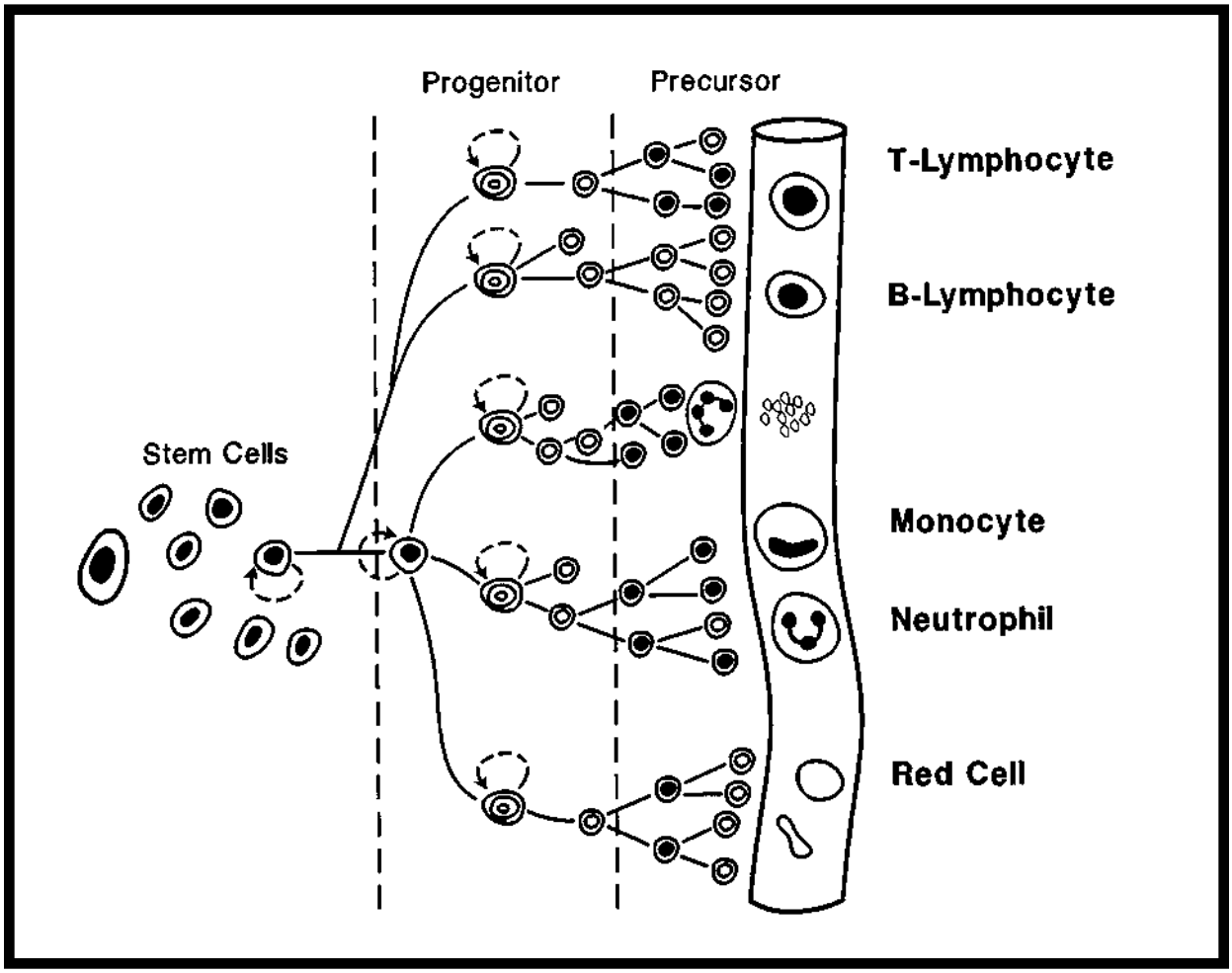

Figure III-12

The hematopoietic stem cell; the target for gene therapy for Cooley's anemia. Blood contains cells of several types, including red blood cells, neutrophils, monocytes, platelets, and lymphocytes. Contained within the bone marrow and lymph nodes are the precursor cells to these blood elements; these precursors actively divide and mature before being released into the circulating blood. Progenitors are more primitive cells with self-renewal capability that ultimately differentiate into precursors in the course of blood formation. Stem cells are the most primitive, multipotent cells capable of extensive self-renewal that have the capacity to restore function of the bone marrow in patients undergoing bone marrow transplantation. Effective gene therapy for thalassemia will require insertion of a globin gene into stem cells with subsequent high-level expression of this globin gene in the precursors that form red blood cells.

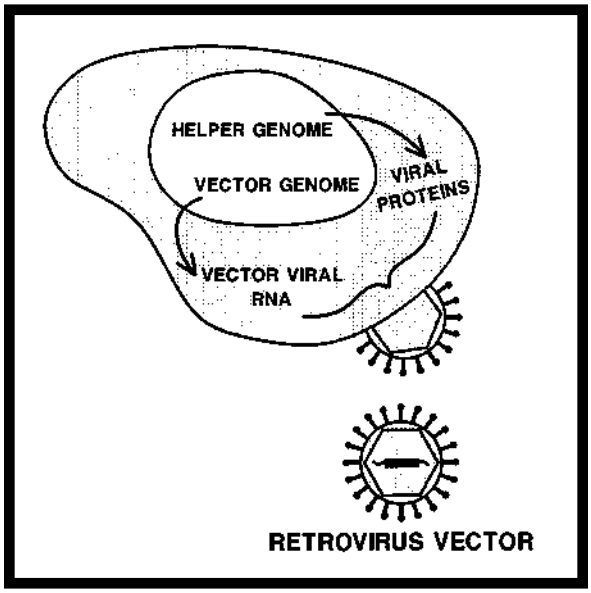

Figure III-13
Retroviral vector producing cells. Genetically engineered cells are capable of producing vectors that will carry a gene into a target cell. The proteins required for vector particle formation are encoded in a "helper genome" whereas the RNA molecule containing the coding sequences for the gene of product of interest is derived from a "vector genome" in the producer cell. Coexpression of these two genomes provide all the components for formation of a vector particle. Because the vector does not carry the coding information for formation of retrovirus particles, the infection cycle ends with insertion of the encapsidated genetic information into the target cell.

required for the virus to recombine with the patient's cellular DNA, and the sequences needed to ensure the adequate expression of the normal gene (figure III-13).

Currently available retroviral vectors can insert genes only into cells that are actively dividing. In humans, as in most other animals, most stem cells are in a quiescent (nondividing) phase of the cell cycle at any given time. Attempts to coax human stem cells into active cycling through the use of cytokines and/or manipulations of bone marrow cells *ex vivo* have not yet succeeded in increasing the frequency and efficiency of gene transfer into stem cells, as measured by the number of genetically modified cells detectable in blood and bone marrow following transplantation.

Retroviral vectors enter cells via transmembrane proteins that act as receptors. Whether the required receptors are expressed on human stem cells is unknown. Researchers are attempting to modify the surface of retroviruses to contain a portion of human protein that will be recognized by, and bind to, the hematopoietic stem cell. This approach may increase the efficiency of stem cell-targeted gene transfer. At the same time, researchers are beginning to explore the use of other types of viruses as vectors for gene transfer. A prime example is adeno-associated virus, or AAV. The advantages of AAV as a potential vector for stem cell-directed therapy are several. This virus does not cause disease in humans. Rather than inserting randomly into the DNA of the target cell (random integration), as is the case with retroviruses, the AAV's genome integrates into a specific position on human chromosome 19. This reduces the risk of unintentional damage to some other essential gene in the process of attempting to correct the targeted genetic defect. Most important is the apparent ability of the adeno-associated virus's genome to express in cells that are not actively dividing. Laboratory research has demonstrated the ability of recombinant AAV's to transfer genes into the more mature progenitor cells, but gene transfer into primitive stem cells has not yet been effectively tested.

Other virus vector systems are also being explored for potential use in stem cell-targeted gene therapy. Human lentiviruses

(which include HIV) can integrate their proviral genome into cells that are not actively dividing. The matrix proteins of these viruses contain a nuclear localization signal that allows the viral nucleocapsid to be transferred into the nucleus of the target cell without the need to break down the nuclear membrane—a process that occurs only during active cell division. The same principles used in developing packaging systems for murine leukemia viruses (figure III-13) may also be applied to the lentiviruses. However, gene regulation in these viruses is more complex, necessitating more intricate and challenging genetic engineering than is needed to produce vector systems based on murine leukemia viruses. Growing understanding of the functions of lentivirus gene products will facilitate development of the needed packaging cell lines.

It may also be possible to develop vector systems that are not based on viruses. One promising approach is the use of synthetic liposomes (microscopic spherically shaped, fatty coated particles) to introduce a normal copy of the defective gene into a cell in combination with a bacterial protein that facilitates insertion of the normal gene into the chromosome in place of the defective one. Liposome-based systems have the potential advantage of offering far greater flexibility in terms of the genetic components that can be transferred to the target cell. Proteins that facilitate integration of the transformed gene by genetic recombination can be included. The development of successful liposome-based approaches ultimately might permit gene correction rather than gene replacement. That is, a copy of the therapeutic gene could be inserted at a specific chromosomal location.

An alternative to gene replacement or gene correction in thalassemia is the use of a transcriptional element to reactivate the quiescent gamma-globin genes, which were turned off during the switch from fetal to adult hemoglobin (figure III-4 B). Many of the transcriptional regulators involved in determining high-level globin gene expression have been identified. Current efforts focus on understanding the precise mechanisms of hemoglobin switching. Several regulatory proteins that determine the balance of gamma-globin and beta-globin synthesis have also been identified. Enforced expression of a transcriptional regulator that activates the gamma-gene might result in sufficient production of fetal hemoglobin to correct the functional deficiency in beta-globin synthesis. Alternatively, diminished expression of a protein that activates the beta-globin genes during postnatal life may reactivate fetal hemoglobin synthesis by freeing the locus control region regulatory elements to interact with the gamma-globin gene promoter. Although this approach is still far from clinical application, the rate of progress provides reassurance that we will eventually gain the knowledge required for molecular manipulation of the hemoglobin switch through gene transfer.

Overall, the prospects for gene therapy for Cooley's anemia seem very good. The complexity of the biological problems that remain to be solved is considerable (e.g., activating quiescent stem cells, developing better vectors for gene transfer, and clarifying regulatory mechanisms). Hence, it is not possible to postulate a timetable for the clinical availability of gene therapy. However, with continued support for the research efforts described here, the goal of nontoxic, curative therapy for Cooley's anemia should be achievable.

Chapter IV
Quality of Life and Social Issues

A. INTRODUCTION

As clinical advances have increased the life expectancy of patients with thalassemia, new psychosocial aspects of the disease have become important. Expanded opportunities now exist for patients to experience a variety of diverse life options. Along with the new challenges presented by these options, patients and their families must still develop strategies to counteract the negative aspects of living with a chronic illness. The changes in the treatment of thalassemia and the increased life expectancy have also altered the way health professionals view the disease and the outlook for patients. Psychosocial issues exist in all aspects of the management of thalassemia and are especially challenging in prenatal diagnosis, treatment of the adult patient, and care of patients who have successfully undergone bone marrow transplantation.

Psychosocial Issues

Public awareness and education campaigns aimed at lowering the incidence of thalassemia have psychosocial implications such as providing screening programs and genetic counseling that are culturally sensitive. The social stigma long attached to thalassemia—for patients and sometimes even for carriers—is a challenge to prevention efforts and treatment. The diversity of ethnic groups requiring genetic counseling, treatment, and education in the United States raises new, culturally specific issues concerning the psychosocial needs of patients.

In the United States, thalassemia major has become a disease primarily of adolescents and adults. As patients mature, several psychological variables may compromise patient adherence to treatment regimens. Moreover, when adolescent patients with poor compliance develop complications of iron overload, medical complications may affect their ability to participate in age-appropriate activities and may foster negative health and psychosocial behaviors.

The increasing age of thalassemia patients has also confronted patients with a myriad of new psychosocial issues such as employment, sexuality, fertility, and marriage. Concerns about mortality continue to be a major source of distress, particularly in adult patients who have lost peers to the disease. These issues should be addressed in conjunction with the medical, developmental, and psychological variables unique to each individual.

The available literature on assessing the psychosocial needs of thalassemia patients is relatively limited. Few studies have addressed the qualitative aspects of living with the disease in adolescence and young adulthood. The paucity of psychosocial research is due in part to the fact that the

young adult patient population has only recently achieved age levels at which investigation into a variety of psychosocial aspects is feasible.

With increased longevity, patients face a constellation of challenges relating to an uncertain future with a chronic illness. The constant life stressors associated with thalassemia and its complications have been shown to have significant effects on the patients' quality of life. A central issue addressed in previous studies is that of self-esteem. Physical deformities that sometimes accompany thalassemia, the label of being a sick child, and the inherited nature of the disease may influence a patient's self-esteem. Research on this subject, however, is limited by small samples and the studies have not determined the effect, if any, of new therapeutic regimens and improved health on self-esteem.

Compliance with Treatment

Compliance with medical treatment of thalassemia, including regular transfusion and iron-chelation therapy, is essential for an optimal prognosis. The impact of psychosocial variables on compliance has been an important area of research. Significant correlations have been found between psychological dysfunction, low self-esteem, poor body image, and noncompliance in patients with thalassemia. Compliance is compromised most often by psychological disorders; oppositional disorder and depression are the most prevalent dysfunctions. In addition, poorer psychiatric adjustment to the disease has been identified in patients with impaired understanding of their illness.

Compliance has been shown to be inversely related to the complexity and the duration of therapy. For patients with thalassemia—particularly adolescents—the perceived benefits of adhering to chelation therapy may be difficult to accept and compliance may be compromised. Education about the disease is a key factor in promoting compliance, psychological adjustment, and positive coping skills. However, education is not enough to promote adherence to a complex medical regimen. Behaviorally oriented programs, combined with education and the inclusion of family members, have successfully improved compliance.

B. A PATIENT'S PERSPECTIVE

(This section was written by a patient with Cooley's anemia)

As an adult thalassemia patient, it seems to me that many physicians and health care professionals often only think about the basic treatment of thalassemia—the bimonthly transfusions and the daily chelation treatment. Perhaps this is because all they can see is the blood and the pump. Even then, many assume or come to believe that the disease is no longer a problem. They think, "You get your blood, use your pump with the drug, and that's that." The scope of the disease and the whole person are forgotten.

Suffering with thalassemia goes beyond the transfusion and chelation therapy. There is a whole person, a human being, attached to that bag of blood or to that pump. Let's look at that other side. Let's see what else patients deal with on a daily basis. What are some of those things that others all too often do not see or even realize exist?

I was raised to believe that this disease is nothing to be ashamed of and that it will be a part of me for the rest of my life. I could not change it, a magic potion would not cure me, and this was something I had

to learn to accept and live with. My family was very open about Cooley's anemia—it was never a secret from anybody. In fact, there were times when I was too open about it. The only time I ever feared telling anyone I had a blood disorder was when transfusion-related AIDS cases were being reported. I was not ashamed of the situation, but rather I feared the reaction of the person I told. Rejection is something everyone must deal with, but my situation was more complex. There are people who will not accept me because of this disease that will be with me until I die. Therefore, I need to move on to people who could accept me completely.

I had to grow up learning that I was different from my family and friends—I was special. I had special blood and special medicine and I did not have to take physical education classes because I was special. How "special" is it to be stuck three and four times with needles for transfusions every other week, to miss school or work, and then work twice as hard to catch up? How special is it to stick myself with a needle every night in order to administer a medicine that burns and irritates? Maybe the special blood I receive will be contaminated with AIDS or hepatitis. How special was I when my classmates teased me because I was a scrawny kid who looked half my age?

I eventually learned it was all because of Cooley's anemia, and at times I did not feel "special." Learning to accept and cope with something I could not control was not easy until I realized that indeed I did have some power over this disease—control of how well I could feel and live. After years of humiliation, lacking self-confidence, feeling inadequate to the human race, I realized that I now needed to become *my* own doctor. I was tired of looking like a kid, tired of always proving myself, and tired of having to defend my every move. After years of fighting, I developed a different outlook. Some may see this as an "attitude," but it's just my way of saying, "I'm tired of the way I've been treated and now it's my turn."

Doctors feel that all they have to do is treat the disease physically—transfuse the patients, chelate the patients, and do the annual check-up. But what about the patients as people? How do they adjust to live a fairly normal life? How can they feel as normal as possible? How do they live a productive life without fearing the unknown? The disease goes beyond blood and deferoxamine. It affects every aspect of the patients' lives. We wake up everyday with this disorder and sleep with it every night. It places boundaries on dreams and creates fear in those of us who suffer from this disorder. Many patients will never admit their fears, but each of us has them deep down inside ourselves.

Growing up was very lonely at times. Even though I went to the clinic with other patients, I thought that I was the only one feeling the way I did. My best friend, who also suffered with thalassemia, was fortunate enough to mature somewhat normally. He was a big support, but there were many days that the desperation was mentally and physically great and destructive. Rebellion became a way of life. Although I wasn't like the healthy children, I could at least act like them and do as much as possible. I look back and regret some of the negative things I did just to "fit in."

It was then that I became involved with the Thalassemia Action Group (TAG), the patient support group sponsored by the Cooley's Anemia Foundation. I finally found the missing piece. Meeting other patients who shared the same emotions,

fears, and concerns as I did produced such a feeling of relief. Loneliness soon disappeared and the feeling of acceptance was overwhelming; I was not alone. It was from other patients that I learned about the different treatments available for all aspects of thalassemia. TAG taught me to take control of my life and care for this disorder. TAG was something I desperately needed for survival.

Patient and parent support is an absolute necessity in the treatment of thalassemia. The family as a unit must be taught about the disease in its entirety. The patient must be encouraged to care for himself or herself as much as possible. Education and awareness, beyond transfusion and chelation therapy, is vital. Physicians must be concerned about the patient's need to grow and develop as a normal adult. They must try to answer the issues about maturing to an adult. These questions are often overlooked or dismissed as minor issues. The patient's fear of AIDS, hepatitis, liver and heart failure, developing diabetes, dying, and even succeeding in life are issues that are often neglected by the health care provider. Physicians and the health care teams need to be aware of patient support systems and encourage participation. Treatment does not end at the hospital. When a patient becomes involved in support groups, a sense of belonging and self-worth are gained. The patient shares knowledge and learns that education and careers can be a real part of his or her life. Dreams of becoming a doctor, teacher, nurse, and a *parent* are then tangible parts of the patient's life.

Physicians and health care professionals should investigate areas in which they can disseminate proper information on genetic screening, counseling, and/or prenatal diagnosis of thalassemia minor and major. Proper referrals to those individuals who can accommodate screening and prenatal diagnoses are a vital part of education and prevention.

More assistance is needed from the hospitals' case management systems to help obtain adequate health care coverage. Today many families cannot obtain proper coverage to adequately treat the patient. The financial expenses incurred by the patient's family can be a burden that often destroys the morale for positive living. Guidance, support, and assistance are needed for the family and patient to assist in making the best health care choices.

The Cooley's Anemia Foundation addressed as many as these problems as possible when it established TAG, the only patient-run Cooley's anemia support group in the United States. Although TAG is funded by the Foundation, it is a self-directed body. TAG offers support to patients and encourages a positive way of living. TAG also promotes compliance with the iron chelation therapy and presents ideas and suggestions to ensure this compliance. The Cooley's Anemia Foundation funds an annual patient-parent conference where leading researchers, physicians, and nurses present current and future therapy for thalassemia. Future treatment for thalassemia may include gene therapy and bone marrow transplantation. The patient-parent conference also serves as a psychosocial session where patients and parents are separated, giving an opportunity for each to speak openly about fears, concerns, and other issues. From this session, patients and parents can develop confidence and gain support from their peers. The feeling of being alone is now an experience from the past. Friendships have been made and maintained forever.

The physicians and health care professionals must continually maintain a positive outlook and should remember that treating the disease goes beyond blood and deferoxamine.

C. A PARENT'S PERSPECTIVE

(This section was written by a parent of a patient with Cooley's anemia)

Many adults who carry the gene for Cooley's anemia are unaware of the possibility of having a child with this disease. They may be unprepared for the intensive home management and care which is an essential part of their child's long-term prognosis.

My general practitioner tested me for the thalassemia trait at age 17. Our family knew that my father was a trait carrier, and we were sensitive to the presence this and other genetic traits in the family. Shortly before our marriage, my wife and I returned to my general practitioner for blood tests. The test performed showed again that I was a trait carrier, but that my wife was not.

Three years later we rushed our second daughter, then six months old, to our local hospital. Earlier in the day while she was having a routine checkup, her pediatrician tested her blood and discovered her hemoglobin measured 7, seriously below the normal 11 to 13 range. As we waited for additional blood to be tested, he informed us that our daughter might be suffering from a serious infection or a blood loss. Late that evening, the local hematologist/oncologist came by to examine her. After the exam, the doctor also felt it was probably an infection but decided to ask us about our family background.

As we explained our family history, I mentioned my thalassemia trait status and the doctor asked if we would bring in the blood work that had been performed on both of us 3 years earlier. The next day there was still no change in our daughter's condition. When we met with the hematologist to give her the blood work, within seconds of reviewing our papers she became very serious and concerned. She showed us that we had very low MCV readings indicating that our red blood cells were smaller than normal. In her experience, this clearly indicated that both my wife and I were thalassemia trait carriers. She then stated that, although it would take several more weeks to confirm our daughter's diagnosis, she was confident our daughter had inherited thalassemia major.

During the past 7 years, our daughter has been transfused with more than *25,000 cc of red blood cells* or about 83 units. She receives this blood approximately every 3 weeks and will continue to do so until a cure is found. In addition, she has been on a strict schedule of daily infusion therapy with the drug deferoxamine to partially remove the excess iron that accumulates in her organs. Over time, this iron has a lethal effect on the patient's organs, potentially destroying the liver and severely damaging the heart.

From a parent's perspective, it is difficult to truly articulate how high maintenance this disease is for the patient and the patient's family. Often when I speak to groups, I try to explain that there is never a day off from Cooley's anemia. What I am trying to convey is that every nightly infusion of deferoxamine must be charted and monitored over time to cancel out as much iron overload as possible from the transfused red blood cells. Mixing the deferoxamine to obtain the proper consistency and inserting

the needle under the skin requires patience and commitment. Often patients develop welts that make the insertion of the needle and wearing of the infusion pump uncomfortable. This becomes a very serious problem because lack of compliance with this drug therapy is frequently the cause of premature death.

One option some parents and patients use to enhance compliance is to have a port surgically inserted into the patient's chest to aid in drug infusion. This decision is a stressful one since a cautious effort must be made to ensure the sterility of equipment and careful placement of the needle. The patient must be careful not to infuse the drug too rapidly for fear that it will arrest the heart. Patients must be concerned with crystallization of the deferoxamine near the passage and the potential for channeling the drug into an artery. There are some patients who have had up to five ports by age 5. Some patients have developed clots. Fortunately, others have used the port trouble-free for years.

As president of the Cooley's Anemia Foundation, my work as a volunteer with this nonprofit organization is to help advance treatment and medical research and to find a cure for this disease. Over the past 40 years, the Foundation has seen brilliant advances and has strong hope for a cure. We are mindful that this disease affects patients in many different ways. Patients die from lack of compliance with drug therapy, heart and liver failure, stroke, chronic hepatitis, and AIDS. The disease also encompasses a range of side effects, particularly diabetes.

As a parent, I do not want to undervalue the family's role in treatment, both medically and socially. Parents of young children must assume primary daily responsibility for their child's care. They must become educated about their child's medical condition. They must arrange schedules to accommodate blood transfusions, to administer of deferoxamine, and to obtain medical supplies. In addition, they may shoulder a financial burden due to lack of insurance and large out-of-pocket expenses.

Furthermore, parents may feel tremendous guilt about being the cause of their child's condition because of the genetic link. They may be anxious about their child's lifespan and quality of life. However, it is important to remind parents and patients that the future is brighter than ever for a child with Cooley's anemia.

An excellent source of information for parents and medical professionals can be obtained through the Cooley's Anemia Foundation. The Foundation can provide medical information, help with insurance problems, and act as a network between those affected and legislators and researchers involved in improving patient care and opportunities. The Foundation can also link families with the Foundation's patient support group, the Thalassemia Action Group, discussed more fully in the previous section.

The Cooley's Anemia Foundation urges a continued and increased investment in research to improve and cure this condition. In the medical area, patients would benefit from the establishment of a clinical network for the treatment of patients with Cooley's anemia and similar hemoglobin disorders. Patients need an oral chelator; a fetal hemoglobin enhancement drug; more research into a genetic cure; and a safe, plentiful blood supply with increased emphasis on decreasing antibodies and identifying new viruses and bacterial contamination. We must continue to seek

improvements in bone marrow transplantation with particular emphasis on the thalassemic patient.

Socially, patients must have access to specialized health care and universal health care. We must work to provide a future where patients do not have to depend on Federal disability status to receive care for their chronic condition. Removing these social barriers will go a long way in sustaining treatment compliance and in helping patients celebrate life.

It has already been established that prior research into this disease has helped to improve other conditions. It is quite reasonable to expect that these advances will continue to occur as more progress is made in improving treatment and finding a cure for Cooley's anemia. We urgently need improved methods to identify and educate all trait carriers who are at risk of having a child with thalassemia. We hope that as *in utero* stem cell transplantation techniques develop, unborn thalassemic children may eventually be cured *in utero*.

In my role as a parent of a child with Cooley's anemia, I have seen the daily struggle for life that other similar patients face. As the president of the only national charity dedicated to helping Cooley's anemia patients and families, I look forward to the day when thalassemia will be a curable disease.

D. FUTURE IMPLICATIONS AND CONSIDERATIONS FOR PSYCHOSOCIAL RESEARCH

Assessing the new and emerging psychosocial needs of the thalassemia patient is both a research and clinical issue. Therefore, assessments of patients and their families should be undertaken to determine psychosocial functioning and to address psychological variables that influence and promote healthy adaptation to the disease. Baseline and annual psychological evaluations will provide important information to practitioners, patients, their families, and the medical community at large. Such information will serve to meet the individual needs of patients and also to establish a platform of data upon which to develop strategies for enhanced care.

The role of future research includes defining and assessing the following: (1) patients perception of the future; (2) perceived needs for psychosocial support; (3) psychological factors in compliance, health promoting behaviors, and longevity; (4) cultural variables in disease containment, patient education, and treatment of new immigrants; (5) vocational issues and expectations; (6) fertility status in patients; (7) sexuality and relationship expectations; and (8) level of patient functioning in school and at work.

Thalassemia is one of several diseases with a prognosis that has changed from death in early childhood to one of chronicity but increased life expectancy. Therefore, a timely and unique opportunity exists to study the dramatic changes experienced in the patient's perception of life in terms of surviving a childhood illness and living with a chronic disease. Findings from such research have potential implications for patients with other chronic illnesses and survivors of diseases that were once considered fatal.

Research in these specific areas, along with early intervention and assessment, can serve to facilitate better patient care, to increase life expectancy, and to improve the quality of life. The benefits of such research also have the potential of reducing the cost of the illness to the patient and to society at large.

Chapter V

Recommendations for Research

Many of the landmark advances in biomedical research can be traced back to basic research in the area of the hemoglobinopathies and especially Cooley's anemia. Research on Cooley's anemia has contributed to a wealth of knowledge in human biology and medicine. Fundamental knowledge about the control of gene expression has resulted from basic research on the thalassemias. Over the years, this has been applied to abnormal gene function in several more common diseases such as sickle cell disease, diabetes, and cancer. The innovative approaches to treatment of Cooley's anemia patients with blood replacement therapy and iron chelation have been applied to more common diseases. The knowledge gained from basic research on gene therapy approaches to the treatment of Cooley's anemia and bone marrow and stem cell transplantation will be directly applicable to the treatment and cure of dozens of genetic and acquired hematologic diseases and disorders of metabolism.

Without a doubt, many of these advances in basic and clinical research can be traced back to research opportunities that emerged as a result of specific initiatives implemented by the NHLBI. These initiatives have included the following Requests for Grant Applications (RFA).

—Enhancement of Fetal Hemoglobin Production (1976-1979)

—Clinical Research on Improved Methods for the Treatment of Beta-Thalassemia Major (1976-1979)

—Further Research on Improved Methods for the Clinical Management of Cooley's Anemia (1979-1982)

—Regulation of the Cloned Human Beta-Globin Gene (1986-1991)

—Iron Overload in Cooley's Anemia (1989-1994)

—Gene Therapy Strategies for the Treatment of Cooley's Anemia (1992-1996)

The remarkable improvement in the prognosis for Cooley's anemia patients in the past 20 years would not have been possible without the leadership role taken by the NHLBI. The Institute is urged to continue this leadership role until improved treatments and a cure are found. The research momentum that has developed during the past several years needs to continue, especially in the following specific areas covering a spectrum of basic, clinical, and psychosocial research.

- **Develop safe and effective orally active iron-chelating agents.** Severe anemia remains the major underlying problem for patients with Cooley's anemia, and combined red blood cell transfusion and iron-chelation therapy will remain the primary treatment for the foreseeable future. The single development that

would most improve the lives of transfused patients with Cooley's anemia would be the development of effective iron-chelating agents that avoid the daily, prolonged intravenous or subcutaneous infusions of deferoxamine that are now needed to prevent disability and death from transfusional iron overload. Studies with the hydroxypyridinone, deferiprone (L1), have demonstrated the feasibility of effective iron-chelation therapy with an orally administered drug, but the risk of neutropenia with this drug may restrict its clinical usefulness. Continued research efforts to develop safe and effective orally active iron-chelating agents or other alternatives to parenteral deferoxamine are urgently needed.

- **Develop a consensus regarding the optimal management of thalassemia intermedia.** Thalassemia intermedia is a nonspecific term used to describe patients who are homozygous, or doubly heterozygous, for mutations within the beta-globin gene that usually result in the disease known as Cooley's anemia. These patients, however, may maintain sufficiently elevated hemoglobin concentrations without regular transfusions. Sometimes, these patients can be shown to have inherited mild mutations of the alpha-globin gene, another form of thalassemia (alpha-thalassemia) that may partially correct globin-chain imbalance or mutations resulting in increased synthesis of fetal hemoglobin, that make this form of Cooley's anemia milder than usual.

  However, there is a wide variability in the problems of patients with thalassemia intermedia. Many patients have only mild or moderate anemia, may attain normal growth and sexual maturation, and enjoy a good quality of life without regular transfusions. On the other hand, some patients may suffer from poor growth, delayed puberty, severe exercise intolerance, leg ulcers secondary to poor tissue oxygenation, and abnormal expansion of the bone marrow often associated with fractures of the long bones or compression of the nerves. In many patients with thalassemia intermedia, symptoms often appear less well tolerated with advancing age. The clinical problems of thalassemia intermedia include progressive fatigue, disfiguring facial changes because of expansion of facial bones, compression of spinal nerves, osteoporosis and fractures of marrow-expanded bone cavities, and progressive iron loading even in the absence of transfusion (due to increased absorption from the gastrointestinal tract). Effective management of such complications may eventually require transfusions, but the risk/benefit of this disease management is still debated. A consensus as to the optimal management of these patients is awaited.

- **Develop approaches to the accurate, noninvasive assessment of body iron burden.** In patients with Cooley's anemia, reliance only on convenient screening tests of body iron burden such as serum ferritin concentrations may over or under estimate the body iron burden of the patient. This is because the serum ferritin concentration is influenced not only by body iron but also by inflammation or infection, ineffective erythropoiesis and red blood cell breakdown, deficiency of ascorbic acid, and hepatitis, all of which are common in thalassemia. Therefore, the assessment of body iron burden in selected patients should include regular determinations of liver iron concentration using tissue from liver

biopsy or noninvasive measurements of hepatic magnetic susceptibility, which provides quantitatively equivalent results. At present, there is no modality that permits the accurate assessment of iron loading within the heart. Because the severity of iron loading within the heart determines the survival of patients with Cooley's anemia, the development of noninvasive methods such as magnetic resonance imaging (MRI) to quantitate iron loading in the heart, which is not accessible to magnetic susceptometry determinations, is urgently needed.

- **Develop safe and effective therapies to enhance fetal hemoglobin production.** Progress in our ability to reverse the hemoglobin switch and turn on fetal hemoglobin has increased in the past decade, giving promise that a new, effective form of therapy for thalassemic patients may soon be available. However, further research is necessary to understand more precisely how these agents induce fetal hemoglobin production, and additional clinical trials are needed to test the effectiveness and safety of these new switching agents as they are identified.

- **Support stem cell biology and transplantation research.** Based on the substantial progress noted in the past several years with transplantation for diseases such as aplastic anemia and early-stage leukemia, it is likely that treatment options and results with hematopoietic stem cell transplantation will continue to evolve and improve. Research in stem cell biology and transplantation opens exciting prospects for safer and wider application of unrelated or mismatched marrow and peripheral or cord blood transplants for inherited diseases of marrow function, including Cooley's anemia. Further research and development of nontoxic, antibody-based pretransplant conditioning regimens would avoid the chemotherapy-associated toxicities. Further clinical research will result in improvements in outcome for older patients and those with advanced stage disease, and the role of stem cell transplantation in the overall management of patients with thalassemia will become more clearly defined. Importantly, with further basic research and clinical development of stem cell transplantation, this devastating disease may someday be safely eliminated by transplantation of genetically corrected autologous stem cells.

- **Expand support for gene therapy research.** Therapies designed to provide a lasting cure for Cooley's anemia would alleviate the life-long dependence upon blood transfusions and iron-chelation therapy. In total, current treatment modalities for Cooley's anemia may cost as much as $100,000 per year. Successful gene therapy would drastically reduce and even eliminate these costs to patients and society. Additionally, the morbidity and mortality associated with this disease would be virtually eliminated.

- **Enhance the safety of the blood supply provided to patients with Cooley's anemia.** In patients with Cooley's anemia who receive hundreds of units of blood transfusions in a lifetime, viral infection remains a serious complication of this life-saving therapy. The most frequently encountered viral infection in patients with Cooley's anemia is hepatitis C, responsible for more than 90 percent of non-A non-B hepatitis infections in transfused patients. With screenings for HIV, hepatitis B, and hepatitis C now under way in the United States, viral infection

may be, in large part, preventable in transfused patients. However, most patients with Cooley's anemia, who received regular blood transfusions before the availability of programs screening for hepatitis C antibody, have evidence of previous infection with hepatitis C virus. The risk of progression of hepatitis C infection to chronic active hepatitis and cirrhosis is not clearly defined but is of serious concern in patients with Cooley's anemia in whom transfusional iron accumulation may aggravate viral damage to the liver. Large-scale programs to reduce the risk of viral transmission in red blood cell concentrates remain a priority for future generations of patients with Cooley's anemia.

- **Develop safe and effective treatment of viral hepatitis.** Hepatitis C virus is the most frequent cause of hepatitis in patients with Cooley's anemia and, as noted, may be more serious in patients with Cooley's anemia in whom the liver may already be damaged by iron overload. As survival is extended because of good chelation therapy, viral-induced liver failure may be expected to become a major cause of morbidity and mortality in patients with Cooley's anemia. It has been estimated that 50 percent of hepatitis C-infected patients without Cooley's anemia will develop chronic hepatitis and that 20 percent of these patients will develop cirrhosis within 10 years. Successful treatment of hepatitis C-induced liver inflammation in patients with Cooley's anemia has been reported recently with the use of recombinant interferon-alpha. Despite this success, recurrence of liver inflammation is common. More effective therapy for this complication of transfusion is of primary importance in the treatment of patients with Cooley's anemia.

- **Investigate psychosocial needs related to increasing life expectancy.** As patients with Cooley's anemia live longer, healthier lives, they are encountering new opportunities, including employment, advanced education, marriage, and child-rearing. For individuals raised with expectations of restricted opportunities due to chronic illness, a more conventional lifestyle may present major challenges. Investigations of adaption to the improved prognosis of Cooley's anemia may help patients improve their overall quality of life—not just their medical well being. Moreover, an understanding of adaption to an increasing life expectancy will be a model for other chronic illnesses in which new treatments are dramatically altering the long-term outlook.

- **Adapt current counseling programs and written materials for multi-cultural application.** Demographic data indicate that patients with Cooley's anemia in the United States represent a wide variety of ethnic and cultural backgrounds. Patients of Italian, Greek, and African-American backgrounds have been joined by those of Southeast Asian, Indian, Pakistani, Arab, and Chinese backgrounds. Counseling and educational materials must be sensitive to the particular needs and concerns of individuals from different cultures. In addition, some basic materials need to be translated into the native languages of recent immigrants who represent an increasing proportion of infants and children recently diagnosed with Cooley's anemia.

- **Develop new approaches to improving compliance with iron-chelation therapy, particularly among teenagers.** Despite the availability of

deferoxamine for more than 20 years, the accumulation of excessive iron remains the major cause of morbidity and mortality in Cooley's anemia patients. Iron-chelation therapy is difficult, painful, and expensive. Moreover, the absence of any immediate or short-term clinical benefit makes the daily therapy an act of faith. Under these conditions, it is not surprising that many patients, especially teenagers, fail to adhere to the prescribed treatment plan and eventually develop congestive heart failure and other iron-induced organ damage. Clinicians with expertise in the treatment of Cooley's anemia need to be brought together with specialists in adolescent medicine and experts in compliance with medical regimens to devise new approaches to chelation therapy and to study the effects of these approaches.

- **Investigate the psychosocial impact of hormone therapy in adolescents and young adults.** During the past decade, hormonal replacement, or supplemental therapy, has become part of the medical management of thalassemia. Hormonal therapy should be evaluated in terms of its psychological impact on self-esteem, body image, social relations, and sexuality. Assessment of the potential benefits of hormonal therapy on the psychosocial functioning of patients should be undertakn.

- **Establish a network of medical centers to enable rapid testing of new clinical modalities.** New therapies are rapidly being developed in the area of iron chelators, fetal hemoglobin enhancing drugs, and gene therapy. Progress in clinical research would be greatly enhanced if these potential new therapies could be rapidly and systematically studied in small and medium-scale clinical trials. This could be accomplished more rapidly, and at a lower cost, if clinical centers were in place and poised to begin clinical investigations. The important psychosocial research recommendations would also be facilitated through such a network of thalassemia medical centers.

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