Technical note 5

Hemoglobin disorders: a case study of genetic disease

Inherited hemoglobin disorders are currently the best studied and defined of all human genetic diseases. They are probably the most common single-gene diseases in the world (Weatherall and Clegg, 1981). Because of that, and because the blood- manufacturing cells in bone marrow are so accessible, hemoglobinopathies were presumed, until several years ago, to be the first candidates for human gene therapy.

Although innovative recombinant DNA technology has pinpointed the genetic defects responsible for different hemoglobin disorders, recent experiments revealing that the regulatory complexities in the manufacture of hemoglobin, and in gene regulation in general, indicate that hemoglobinopathies will not be effectively treated until these processes are better understood and can be controlled (Anderson, 1984).

NORMAL HUMAN HEMOGLOBIN

All normal human hemoglobins are composed of two pairs of identical protein chains, forming a "tetramer". Hemoglobin differs between the embryo, fetus and postnatal human because genes coding for different protein chains are activated progressively during development. Fetal hemoglobin (HbF), for example, is composed of two alpha and two gamma chains while the adult version contains two alpha and either two beta (95 percent) or, much less commonly (3 percent), two delta chains (Orkin and Nathan, 1981). **These complex regulatory changes in hemoglobin synthesis aid in transporting oxygen across** the **placenta, from mother to fetus. This is possible because embryonic and fetal hemoglobins have higher oxygen affinities than normal adult hemoglobins.**

The two major types of single-gene hemoglobin diseases are sickle cell anemia and the thalassemias.

Table 1.—Human Hemoglobins

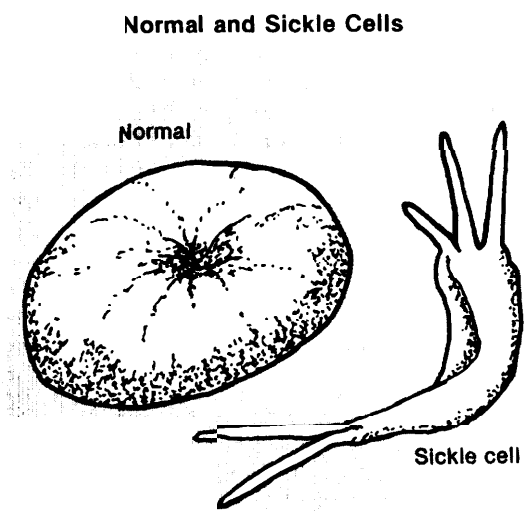
Type of hemoglobin	Chain composition	When present
Hb A	$\alpha_2\beta_2$	Adult life (~95%); small amount during fetal life
Hb F	$\alpha_2\gamma_2$	Fetal life (predominant); adult life (~1-2%)
Hb A_2	$\alpha_2\delta_2$	Adult life (~3%)
Gower-1	$\zeta_2\epsilon_2$	<12 weeks gestation
Gower-2	$\alpha_2\epsilon_2$	<12 weeks gestation
Portland	$\zeta_2\gamma_2$	<12 weeks gestation

SOURCE: Orkin, S. H. and Nathan, D. G. "The Molecular Genetics of Thalassemia" In: H. Harris, A. Hirshorn (eds.) *Advances in Human Genetics* (New York: Plenum Press, 1981) vol. 2, p. 233-288.

These genetic defects are not located on a sex chromosome and usually require two faulty copies of the gene for the disease to manifest clinically. Hundreds of thousands of people have only one faulty copy of the gene out of the allotted two, and thus are labelled heterozygous carriers of these diseases. An estimated 200,000 people with hemoglobinopathies are born annually, divided equally between sickle cell anemia and thalassemia (WHO, 1982). The thalassemias are most common in Asia, and sickle cell is most common in Africa. Among American blacks, about 8 percent carry sickle cell trait and one in 500 newborns have sickle cell disease (Stern, 1973; McKusick, 1983; Bowman, in personal communication, 1984).

Sickle cell anemia

Sickle cell anemia involves a variation in hemoglobin structure due to substitution of one nucleotide on the beta globin gene, leading in turn to a substitution of the amino acid glutamate for valine (the normal sixth amino acid on the beta globin chain) when the faulty gene is '(transcribed" and used to produce hemoglobin protein in the bone marrow cells. The hemoglobin containing the faulty beta globin chains (Hbs) is less soluble than normal hemoglobin and, under reduced-oxygen conditions, can form a crystal that distorts the red blood cells into shapes resembling



Red blood cells–normal and sickle cell

SOURCE: Office of Technology Assessment.

sickles. These misshapen red blood cells are rapidly destroyed and become lodged in capillaries, leading to partial or total blockage of blood supply to parts of the body. Pain and local-tissue damage results, especially in those organs with extensive capillary networks such as the lungs, heart, kidneys, brain, spleen and hips.

The blood of a person with two copies of the "sickling" gene consists primarily of HbS; this person is said to have "sickle cell disease" and generally has an abbreviated lifespan. The impaired circulation can lead to anemia, pain in the joints, sporadic abdominal pain, lung and spleen damage, ulcerations of the lower extremities and acute episodes such as stroke, kidney failure, and heart failure (Bowman and Goldwasser, 1975). The clinical symptoms are extremely variable, however, and some people may remain completely free of serious illness. A person who possesses one faulty and one normal copy of the beta globin gene has "sickle cell trait" and has blood containing 20 to 40 percent HbS. Such "carriers" typically exhibit little or no clinical symptoms of anemia and have a normal life expectancy.

Thalassemias

The thalassemias are characterized by decreased production of certain hemoglobin chains, There are several types of thalassemia named according to which globin chain is deficient. For example, in alpha thalassemia, little or no alpha globin is produced. The same holds true for beta thalassemia, where little or no beta globin is produced. These are the most common forms of thalassemia.

The decrease in globin production by the affected gene ranges from none at all (as in alpha^{o}- or beta^{o}-thalassemias) to somewhat less than normal (as in alpha^{+}- or beta^{+}- thalassemia). The clinical signs and symptoms are extremely variable, especially among heterozygotes, ranging from none to serious anemia. Generally, the symptoms afflicting an individual heterozygous for thalassemia are exacerbated under physical stress. Prolonged stress can exhaust the auxilliary blood production mechanisms that are already being pushed to maintain normal hemoglobin levels.

The genetic defects underlying thalassemias are as varied as the associated clinical symptoms. The impaired synthesis of globin chains could result from mutations grossly affecting the structure of a globin gene, decreased transcription of the gene, abnormal RNA processing, or defects in the activity and translation of the mature RNA (Treisman, Orkin, Maniatis, 1983).

ALPHA THALASSEMIAS

Most alpha thalassemias involve gene deletion. "Silent carriers" (those showing no clinical symptoms) have one of the four normal alpha globin genes per cell deleted. Those with alpha thalassemia "trait" have two genes deleted and usually show no anemia. "Hb H" disease is associated with the deletion of three genes (Kan, et al., 1975) and is characterized by mild to moderate anemia. Homozygous alpha thalassemia involves deletions of all four gene copies and results in severe anemia, accumulation of body fluid, and intrauterine death (Orkin, 1978).

BETA THALASSEMIAS

There are only two beta globin genes in the normal human genome. If only one copy of the gene is affected, an individual is said to be heterozygous and have beta thalassemia trait. Such heterozygotes are usually asymptomatic, except for occasional mild anemia or slight spleen enlargement. If both genes are affected, the individual is homozygous and has the disease beta thalassemia. Symptoms of the beta thalassemia disease include severe anemia, enlargement of the spleen, liver and heart, skeletal deformation, abnormal facial features, and abbreviated life span.

Other less common forms of thalassemia involve persistence of fetal hemoglobin, however, and therefore constitute models for the study of the mechanisms responsible for switching from fetal to adult hemoglobin synthesis during development. If better understood, this process might be exploited clinically as a treatment for beta thalassemia in which fetal hemoglobin synthesis could be "turned on" to compensate for deficient adult beta globin synthesis.

Other unstable hemoglobins

There are dozens of mutant types of globin protein that replace either the alpha or, more commonly, the beta chains in hemoglobin (Winslow, 1983). Many of these form unstable hemoglobins that deteriorate rapidly and cause anemia. Most are extremely rare, with the exceptions of hemoglobin SC disease and hemoglobin S-thalassemia. These two disorders are hemoglobinopathies that occur in patients who have one sickle cell gene combined with another mutant gene–for globin C in one case and for thalassemia in the other.

The unusual hemoglobinopathies vary widely in clinical severity. Most are relatively well understood. Gene therapy for most of them would involve the same steps in replacing defective genes in bone marrow cells with their normal globin gene counterpart.

Diagnosis of hemoglobinopathies

Because the symptoms of the hemoglobinopathies are very heterogeneous, a definitive diagnosis usually requires assays for abnormal hemoglobin or DNA analysis. The simplest and most common method of diagnosing sickle cell trait and anemia postnatally is through protein electrophoresis of a blood sample (see diagram). Because of the risk associated with fetal blood sampling, however, this procedure may soon be displaced in *prenatal* diagnosis by the less risky procedure of DNA analysis of cells obtained through amniocentesis or chorionic villus biopsy (see app. A).

Electrophoresis can also be used to detect most forms of thalassemia postnatally, except for the "silent carrier" form of alpha thalassemia which may now be diagnosed using restriction endonuclease DNA analysis (Embury, et al., 1979). Prenatal detection of homozygous beta thalassemia has been possible since 1974 through quantitation of the amount of beta globin manufactured by a fetal blood sample (Kan, 1977; Alter, 1979). Prenatal diagnosis of certain forms of beta thalassemia is also possible using DNA analysis (Alter, 1981; Antonarakis, 1982; Boehm, 1983; Connor, 1983; Estein, 1983; Hodgkinson, 1984; Orkin, 1982, 1983; Pirastu, 1983).

Treatment of Hemoglobinopathies

Currently, clinical treatment of hemoglobinopathies is limited largely to treatment of infections, mitigation of the associated symptoms (e.g., pain in the joints), and organ-specific therapy (Dean and Schecter, 1978). There is no effective long-term treatment for sickle cell anemia, and the two treatments available for thalassemia are only partially effective, with undesirable side effects (Adamson, 1984). The first treatment involves repeated transfusions with normal red blood cells can alleviate some of the symptoms, but eventually leads to toxic iron overload. The second treatment, bone marrow transplants, or the transfer of healthy bone marrow from a relative into the patient, has been used successfully to treat homozygous beta thalassemia. This carries a high risk of failure, however, and the possibility of an immune reaction of the patient against the transplanted marrow.

It could be argued that prenatal diagnosis obviates the need for postnatal treatment. However, there will always be children born with hemoglobinopathies and other genetic diseases because: 1) parents often do not realize that they are carriers until they have had an affected child; 2) parents who know they are carriers may chose to take the risk of their child having a genetic disease; 3) prenatal diagnosis is often unacceptable for moral, ethical, religious, or personal reasons; and, 4) genetic mutation is constantly reintroducing defective genes.

Several alternative treatments are currently being developed experimentally that may be divided into three categories: 1) drug therapy, 2) gene therapy, and 3) bone marrow transplant (Desnick, 1981). To date, no form of gene therapy and only a handful of the drug therapies have progressed to the point of clinical trials, and bone marrow transplant appears to be of possible use for only a small percentage of patients.

DRUG THERAPY

Two types of drugs are currently being developed to treat hemoglobinopathies. One type is designed "turn on" the synthesis of fetal hemoglobins to compensate for the faulty or insufficiently produced adult hemoglobins. Some of these drugs are already being tested clinically (Dover, 1983, 1984). The second type is meant to suppress the polymerization or gelling of the sickle hemoglobin molecule that distorts the red blood cells. Some of these drugs have also been tested clinically (Bookchin, 1976; Dean, 1978; Lubin, 1975; Nigen, 1974).

GENE THERAPY

Treatment of hemoglobinopathies through gene therapy, or the insertion of normal globin genes into the embryo (germ line) or into bone marrow (somatic) that is then implanted, is still entirely in the experimental stage in animals. The success rate of in vitro germ-line transplants is still disappointingly low (see app. B). The lack of animal models for hemoglobinopathies has effectively hindered both germ-line and somatic-cell experiments. Recently, however, a model for beta thalassemia was developed in the mouse (Skew', et al., 1983). Even given such models, however, the researcher is faced with the task of having the gene express at all, at adequate levels, at the right time, and in the right tissues in the whole animal.

BONE MARROW TRANSPLANT

Gene transplant for hemoglobinopathies attempts to take advantage of the relative accessibility of human bone marrow cells, where hemoglobin is produced. Bone marrow is removed from a donor who produces normal hemoglobin, who has been matched for tissue compatibility with the recipient patient who suffers from a disorder of hemoglobin. The donor patient then receives radiation treatment sufficient to destroy the cells of his own bone marrow. Once accomplished, the patient receives the transplants of the donor's bone marrow. The recipient is then treated with drugs to

suppress his or her immune reaction against the donated cells (but this also affects general body defenses). If not rejected by the host, the transplanted bone marrow begins to manufacture normal hemoglobin. The procedure is quite stressful to the patient, relatively risky, and not all patients can be matched with compatible donors.