NIDDK Hematology Program Review meeting
March 3, 2005
Marriott Suites at Democracy

The meeting began at 10:00 a.m. with a call to order from the Chair, Dr. Stanley Schrier and a welcome statement from the Director of the Division of Kidney, Urologic, and Hematologic Diseases of the National Institute of Diabetes & Digestive & Kidney Diseases, Dr. Josephine Briggs.

I. Description of Existing Program:

The NIDDK's Hematology Program emphasizes a broad approach to understanding the normal and pathologic function of blood cells and the blood forming (hematopoietic) system. Major areas of interest include diseases such as sickle cell anemia, thalassemias (for example, Cooley's anemia), aplastic anemia, iron deficiency anemia, hemolytic anemias, and thrombocytopenia.

The history and legislative chronology of the NIDDK Hematology research program was delivered by the Deputy Director of NIDDK, Dr. Griffin Rodgers. The basic research emphasis of the program focuses on:

- Blood and blood-forming organs
- Hematopoiesis
- Red Blood Cell metabolism
- Globin molecular genetics
- Disorders , such as Sickle Cell Disease, Cooley's anemia (thalassemia), and Hemochromatosis
- Translational research on iron chelators

A. Blood and Blood-forming Organs

Hematopoietic Stem Cells: It has been known for decades that hematopoietic stem cells (HSC) reside in the bone marrow in a quiescent state and replenish the supply of terminally differentiated cells with diverse biologic functions of the peripheral blood throughout the lifetime of an individual. No other adult cell type retains the capacity for such immense proliferation and differentiation. However, there are large gaps in our understanding about the cells, microenvironment or factors that regulate their primitive state or that control their activation.

B. Hematopoiesis

The NIDDK supports fundamental research on the gene expression, cell biology, differentiation, and functioning of all of the blood-forming (hematopoietic) cells, including the adult HSCs. A major research initiative, launched in 2002, is the Adult Hematopoietic Cell Lineage Genome Anatomy Projects (HCLGAP). The purpose of the HCLGAP is to identify the gene expression profiles of hematopoietic stem cells and other cells in the hematopoietic lineage in health and disease. Investigators in the

HCLGAP are charged with developing protocols and reagents for characterizing cells in the HSC lineage, and characterizing gene expression patterns in these cells using advanced technologies and bioinformatics techniques. Data generated by the HCLGAP is being freely shared with the research community in order to fully capitalize on this initiative.

C. Red Blood Cell metabolism

Erythropoiesis: Erythropoiesis is the generation of mature, oxygen-carrying red blood cells from blood-forming cells called erythroid progenitor cells. Defects in erythropoiesis, which can have a variety of causes, can result in anemia. While there are dozens of causes of anemia, not all of them interfere directly with red blood cell production, such as blood loss due to gastrointestinal or excessive menstrual bleeding. A few common conditions and diseases leading to defective erythropoiesis and consequent anemia include:

- Vitamin and mineral deficiencies such as iron, folic acid or B12 deficiency.
- Primary bone marrow failure--for example, aplastic anemia.
- Excessive blood cell destruction because of:

-Abnormal red blood cell enzymes (for instance, glucose-6-phosphate dehydrogenase (G6PD) deficiency, which is especially common in African-Americans)

-Abnormal assembly of red cell membrane proteins (for instance hereditary spherocytosis and hereditary elliptocytosis)

-Abnormalities of the globin molecule, the protein part of the hemoglobin molecule, which carries oxygen in the red blood cell (for example, sickle cell anemia)

-Abnormalities in production of the protein portion of the hemoglobin molecule (for example, thalassemia)

-Abnormalities of the immune system (for example, immune hemolytic anemia)

- Chronic infections or inflammation.
- Kidney disease, leading to reduced erythropoietin production.
- Toxins, certain medications and radiation.

The NIDDK supports research on several aspects of erythropoiesis, including its regulation by the hormone erythropoietin, produced by the kidneys, gene expression in erythroid cells at distinct stages of erythropoiesis, and membrane biology of cells in the erythroid lineage. Aiding research in this field, investigators in the NIDDK intramural program have developed and are maintaining “Hembase,” a World Wide Web based resource for scientists studying genes expressed in erythroid cells during red blood cell production.

D. Globin molecular genetics

As hematopoietic cells differentiate into mature erythroid cells, the primary event that occurs is production of hemoglobin. Stimuli for production of the necessary amount of hemoglobin exist throughout the body, primarily as oxygen sensors. Efficient production of hemoglobin in response to a variety of environmental conditions is essential to avoid anemia. Systems biology approaches are necessary to identify, describe, quantitate and piece together the molecular events that are involved in

responses to rapidly changing physiological and environmental conditions and more long term, developmental changes.

Hemoglobin production is the result of synthesizing two different proteins, alpha and beta-globin and the production of heme, itself the end product of an eight-enzyme biochemical pathway, in stoichiometric quantities. When there is an imbalance in any of these three entities, anemia may occur. The study of globin synthesis is not only critical to our understanding of the underlying mechanism of hemoglobin production, but it also serves as a model system with which to understand gene regulation in a variety of settings.

The NIDDK supports research on the primary genetic structure of the globin gene macro-locus, transcriptional factors involved in transcribing the locus at different stages of development, chromatin structure supporting and facilitating maintenance, transcription, DNA replication and position in the 3-dimensional realm of the cellular nucleus.

E. Disorders

In accordance to the National Cooley's Anemia Control Act (PL 92-414) and the National Sickle Cell Control Act (PL 92-294) and with subsequent appropriations bills, the NIDDK has supported research in the basic etiologies of sickle cell anemia, thalassemia, aplastic anemia, iron deficiency anemia, hemolytic anemias, and thrombocytopenia.

Gene Therapy: The possibility of curing inherited diseases by replacing a defective gene with a gene that functions properly has tantalized scientists for more than a decade, and patients with certain forms of anemia, such as Cooley's anemia, are likely to benefit from the considerable efforts under way to develop gene therapy technologies. How to "package" an affected gene, deliver it to the proper cells, activate it, and ensure appropriate and sustained manufacture of its product are questions being addressed by current research efforts.

F. Translational Research on Iron Chelators

Iron Metabolism: Iron is an indispensable element in oxygen uptake and transport by red blood cells. The majority of body iron is bound up in hemoglobin in the red blood cells, and normal iron balance is maintained primarily by controlling dietary iron absorption in the intestine. Pathologies that interfere with iron uptake or utilization by red blood cells can lead to anemia. Basic studies of iron absorption, its transport between tissues, and its storage are supported.

With the cloning of the major mammalian iron transporters, it is likely that in the near future human mutations will be identified for these transporters. In addition, ongoing studies on gene modifiers of iron metabolism are likely to provide several additional genes that may affect disease phenotype and response to therapy.

Iron Chelators: Currently, iron removal is achieved through administration of "iron chelators," drugs which bind to excess iron and enable it to be excreted. Studies supported by NIDDK have led to a better understanding of how the different iron

chelating drugs remove iron from body tissues. This, in turn, has led NIDDK-supported investigators to start testing whether "smart" combinations of chelators may both maximize iron removal and enable use of lower doses of the drugs; early results are encouraging.

Non-invasive Imaging of Body Iron Stores: The "gold standard" for ascertaining excess body iron is through a liver biopsy, a painful, risky, and invasive procedure. Non-invasive imaging approaches to measure iron stores can contribute greatly to the effective clinical management of patients with diseases of iron overload. The NIDDK Hematology Program has been at the forefront of developing new technologies for the non-invasive measurement of body iron.

II. Gaps in existing portfolio

Dr. Schrier went around the room and asked each member of the committee to comment on deficient areas or gaps in the hematology portfolio.

A. Iron Chelation

The Cooley's Anemia Foundation has been instrumental in promoting research on thalassemia major. However, other forms of thalassemia and hemoglobinopathies are becoming more prevalent in the U.S., particularly in areas where there have been significant increases in immigration from S.E. Asia. Hemoglobin E, most prevalent in S.E. Asia, is now on the increase on the West Coast, carried either in heterozygous or compound heterozygous state with β-thalassemia. Transfusion induced iron overload and the need for chelation therapy is on the increase for this patient population. Furthermore, as transfusion therapy to reduce the incidence of strokes is being implemented for sickle cell disease, iron chelation therapy is of great importance to this group of patients as well. Thus, the need to continue and improve iron chelation therapy is crucial to offset significant anticipated needs during the coming decade.

In addition, iron chelation therapy is now being used in the treatment of hepatitis, thus underscoring the importance of continuing to develop effective iron chelation therapy.

B. Red Blood Cell Metabolism

Steps need to be taken now to prevent the loss of the vital area of research dealing with red cell metabolism and red cell membrane function. With less emphasis in this field, it may become necessary to reinvent it, wasting a tremendous amount of research dollars and investigators' effort.

Critical components of the RBC remain uncharacterized. For example, crystal structures need to be solved for the various RBC membrane proteins. As many if not all of the red cell membrane proteins are expressed in kidney, pancreas, liver and intestine, the structural information garnered could be widely applicable to our understanding of pathophysiology of a number of human disorders relevant to the mission of NIDDK.

C. Structural Biology

It was perceived that there is a lack of structural work and of detailed understanding of molecular mechanisms of mRNA processing. Thalassemia, for example, may be viewed as a disease of mRNA processing and a better understanding of RNA splicing, editing, stability, degradation and translation is needed. Both the structural data and detailed understanding of mRNA processing are prerequisites to intelligent design for new drugs. There is also a significant concern that a decline in grants using physical chemistry and structural biological methods will erode the talent pool.

D. Hematology Training

A number of issues were discussed regarding the training of young hematologists to become productive researchers. Concerns were expressed that hematology might not be attracting the most talented young investigators. The committee felt it was important to track the individuals being trained on Hematology Institutional Training grants (T32s). What are the outcomes? Do the trainees stay in hematology research? With high quality investigators, exciting advances will be made. Are the T32 grants providing the necessary training for these individuals to become established independent investigators? Effective mentoring of young investigators is on the decline lately with increasing pressures on senior investigators' time to continue their own research programs. This causes an additional "squeeze" on the pipeline of young investigators.

A gap is perceived in training mechanisms for the M.D. researchers to make research contributions. A trainee supported by an Institutional Training Grant, for example, may not acquire sufficient research skills *and* generate enough data with which to write a competitive Mentored Career Development Award (K) application. Support for these individuals needs to be provided so they don't "drop out of the pipeline". With increasing emphasis on translation research there is a critical need for this pool of investigators to be part of future research enterprise.

III. Solutions to the gaps

A. Centers for Hematology Research

There was general agreement that more the Hematology Centers have been very valuable, and a consensus of the external advisors that, if resources are available, the creation of additional Centers would strengthen the field. . A positive effect from the existing Centers has been felt in the community and such Centers create a nucleus for Hematology research. There have been longstanding effects from the core laboratories created by support through the Centers of Excellence program and the development of such infrastructure was a good investment. These Centers may be necessary to advance the field and avoid a stagnation of the field. Perhaps they could also be used to support training at the predoctoral and/or postdoctoral stages of career development in order to expand training opportunities for residents or postdoctoral fellows.

B. Use the Red Blood Cell more effectively as a model

1. The red blood cell is an ideal biological model in which to study oxidative and anti-oxidative mechanisms, in part because of the relatively high concentration of sequestered iron. There should be more research stimulated in this area of research. The results of such studies in the red cell may be applicable to other systems, for example, investigation into the use of antioxidant mechanisms in diabetic complications to minimize the effects of reactive oxygen species.
2. Metabolic profiling or protein modification profiling performed using high throughput analyses needs to be accomplished. It is possible that red cell metabolomic studies will not only revive the field of red cell metabolism, but also be used as a diagnostic tool for abnormalities, stresses or perturbations from "normal" whole body physiology. Monitoring changes in red cells have already proven to be very effective. For example, increased glycated hemoglobin in diabetes, carboxyhemoglobin increases with smoking, abnormal lipids accumulation in the red cell membrane in liver disease. It is very likely such approaches will continue to be highly valuable for a variety of other disorders, including uremia.
3. The red cell and its progenitors provide a valuable biologic system in which to study coordinated gene regulation, interactions between biochemical pathways and systems biology. RNA transcripts are easily purified from erythroid cells and when purified from reticulocytes, there is no confounding contamination from DNA. This allows a more straightforward interpretation of gene regulation, in particular, mRNA stability and decay.
4. Differences between embryonic development and adult differentiation are appreciated using the red blood cell system. Using zebrafish, the first red blood cell "born" may be observed during the development of an organism. Its characteristics are distinctly different from the billions of red blood cells produced daily in an adult organism. What markers might be used to characterize these differences? How might these different features help us extrapolate to differences and similarities between embryonically-derived stem cells and stem cells derived from adult tissues?
5. In diseased conditions that cause premature red cell death, what is the spectrum of conditions that cause this? Are there ways to circumvent the eventual iron overload that occurs with ineffective erythropoiesis?

C. Hematopoietic Stem Cells and their Niche

Reconstruction and more complete definition of the hematopoietic niche is necessary to understand its maintenance properties, cytokine production abilities and role of cell-cell and cell-extracellular matrix interactions that regulate the number of HSCs. Abilities to manipulate the niche will have obvious therapeutic potential.

Within the HSC, a more thorough understanding needs to be achieved regarding environmental cues, receptors, signaling pathways, transcriptional regulation, post-transcriptional, translational and post-translational processes, including trafficking of HSCs and how these processes contribute to the function of hematopoietic stem cells and the lineages derived from them.