Blood: New designs for a new millennium

Whether you believe that January 1, 2000, marks the beginning of the new millennium, this issue of the journal surely represents a major milestone for Blood and the American Society of Hematology (ASH). With this issue, we begin self-publishing the journal, an event we mark by a clean new design. For the past 54 years, commercial publishers, most recently W. B. Saunders Company, ably handled Blood. We sincerely thank the many individuals at Saunders for their outstanding service over the years, helping to make the journal a premier source for basic and clinical research in hematology. The decision to self-publish was not made out of dissatisfaction with Saunders or the journal but, rather, reflects the society’s belief that self-publication will offer the editors greater flexibility in providing the scientific and clinical community with the best that modern hematology has to offer. With the establishment of an ASH publishing office, expertly handled by Sabine Beisler and her staff, and the ongoing exemplary work of the Blood editorial office, headed by Ken Kornfield, we look forward to continuing to report on innovations in biomedical sciences and clinical care into the third millennium.

As we embark on these transitions, it is a fitting time to appreciate the many achievements in the field of hematology, accomplishments that provide a sense of great optimism for the future. Most agree that the beginnings of hematology date to the critical technical advances in the microscope in the 17th century. Red cells were readily apparent in Leeuwenhoek’s improved microscopes, but discovery of the oxygen-carrying function of hemoglobin awaited the observations of Hoppe-Seyler in the 19th century. Leukocytes were probably also apparent to Leeuwenhoek as “colorless corpuscles,” but it was not until the work of Metchnikoff in the late 19th century that their role in host defense was identified. Due to their smaller size, platelets were not identified until Addison described “loose or independent molecules” in the blood in 1842—cells later termed the “dust of the blood”—and it took James Homer Wright to describe their origin in marrow megakaryocytes in 1906. Thus, by the turn of the last century, the major blood cell types were described, their origins identified, and their general functions determined. Wintrobe has identified, and their general functions determined. Wintrobe has

Hemoglobin

A comprehensive list of the molecular accomplishments of any discipline of medicine is beyond the scope of a celebratory editorial, but several developments can be cited as superb examples of how hematology has provided important paradigms for molecular medicine. The biochemistry of hemoglobin provides a stunning example of how the study of blood can lead to a thorough understanding of disease and begin to make inroads into its control. The oxygen-carrying protein hemoglobin was discovered by Otto Funke in 1851 and its reversible oxygenation described a few years later by Felix Hoppe-Seyler. Max Perutz determined the crystalline structure of the molecule, for which he received the Nobel Prize for Physiology and Medicine in 1957. James Herrick was the first to report, in 1910, that some patients with severe anemia have “sickle-shaped” red corpuscles, cells previously recognized in the blood of deer. The abnormal electrophoretic mobility of hemoglobin S was described in 1949 by Linus Pauling, and the characteristic Glu to Val mutation at the sixth position of β-globin was identified by Vernon Ingram in 1957. With the sequencing of normal, sickle, and thalassemic globin genes, the first human diseases were described at the nucleotide level. The myriad genetic mutations that can lead to a single phenotype, such as β-thalassemia, have also taught us valuable lessons on the microheterogeneity of human disease and have provided the model on which most successful screening strategies for genetic disease are based. Such approaches have already yielded important dividends: The introduction of prenatal DNA screening has nearly eliminated new cases of β-thalassemia in many populations.

Dissection of the β-globin gene has yielded other important paradigms for cell biology and targets for novel therapies of hematologic disease. Although of little or no clinical consequence when inherited alone, coinheritance of hereditary persistence of fetal hemoglobin (HPFH) with homozygous β-globin alleviates the clinical severity of sickle cell disease. This and other observations fueled intense study of β-globin gene expression, a pursuit yielding the first example of a “locus control region,” a collection of genetic regulatory elements that affect gene clusters from afar. The desire to pharmacologically mimic HPFH in patients with sickle cell disease led to our understanding that cytotoxic agents enhance γ-globin expression, ultimately leading to successful clinical trials of hydroxyurea in this disease. Although the precise mechanism by which hydroxyurea reduces painful vaso-occlusive events in patients with sickle cell disease may not be entirely dependent on alterations in γ-globin gene expression, it is clear that, for the first time, physicians have available an intervention that can reduce the severity of the disease.
Hematopoietic growth regulators

The study of soluble and membrane-bound regulators of hematopoietic cell growth and differentiation in normal, inflammatory, and malignant hematopoiesis and their application to clinical medicine has provided important paradigms in the regulation of cell production, determination of cell fate, and mechanisms of signal transduction. Translation of this research to the clinic has also provided a means of stimulating blood cell production and function for therapeutic benefit.

Carnot was the first to demonstrate that a humoral substance (first termed hemopoietine) is responsible for the regulation of erythropoiesis.14 Erythropoietin (Epo) was purified from the urine of patients with aplastic anemia in 1977,15 and the molecule was cloned in 1985 by scientists at the Genetics Institute16 and Amgen.17 The cloning and characterization of hematopoietic growth regulators perhaps best exemplify the cooperation possible between academic medicine and the biotechnology industry. In fact, of the 32 recombinant proteins currently licensed for use in humans in the United States in August 1999, 20 are targeted to hematologic disorders, and 12 affect hematopoietic cell growth or function (Epo, granulocyte colony-stimulating factor [G-CSF], granulocyte-macrophage [GM] CSF, interleukin [IL]-2, IL-11, interferon [IFN]-α, IFN-β, IFN-γ, and monoclonal antibodies to CD3, IL-2Rα, tumor necrosis factor [TNF]-α and the TNF-R). The renal source of Epo production was first appreciated by Jacobson in 1957, and the hormone was first used in patients with the anemia of renal insufficiency.18 More recently, Epo therapy has proven valuable in a number of additional clinical settings.19 In a similar fashion, cytokines that stimulate neutrophil, monocyte, and mega-karyocyte production have been identified, characterized, cloned, and tested in patients. These cytokines have also provided important therapeutic advances in patients with other cytopenias (eg, G-CSF and GM-CSF in neutropenia20,21 and IL-11 and thrombopoietin [Tpo] in thrombocytophenia22,23) and in several other clinical conditions (eg, G-CSF and Tpo in stem cell mobilization20,24 and M-CSF in infectious diseases25). Other cytokines that affect marrow-derived inflammatory cells, such as TNF and IL-6, have also been determined to play important roles in numerous disease processes. This understanding has led to additional therapeutic advances. For example, monocyte-derived TNF-α affects neutrophil activation and contributes to the joint pathology characteristic of rheumatoid arthritis; neutralizing reagents to this inflammatory mediator have been shown to ameliorate several refractory clinical disorders.26 The antiproliferative properties of the IFNs have also been identified and exploited; the successful therapy of chronic myelogenous leukemia (CML) or hairy cell leukemia with IFN-α27 provides additional examples of our entry into the age of rational medicine. Furthermore, the availability of these growth factors and their receptors has provided important insights into several aspects of cellular physiology. A number of diseases have been linked to growth factor or growth factor receptor excess or deficiency (Table 1). Finally, a growing understanding of the intracellular signaling pathways employed by hematopoietic cytokines is beginning to provide additional targets for manipulating blood cell production. Studies of the IFN, hematopoietic cytokine, and protein tyrosine kinase families of blood cell receptors have given us JAKs, STATs, and recently, SOCS,37,38 and have expanded the realm of many previously recognized kinases (PI3K, MAPK, PKC) in intracellular signaling. A thorough understanding of the elaborate circuitry that conducts extracellular signals for cell growth, differentiation, and death will almost certainly provide new targets for therapeutic intervention. The clinical success of a rationally designed kinase inhibitor against tumors bearing aberrant kinases39 is the first example of such signaling therapy.

Cytogenetics and cancer

For many years, evidence for a genetic role in malignant transformation was based on studies of animal tumor viruses and chromosomal changes seen sporadically in rare cancers. That altered genes cause most or all human cancers was suspected but awaited formal proof. The description of a signature chromosomal change in CML by Nowell and Hungerford in 1960 (the Ph1 chromosome40), the discovery of the “missing link” between viral oncogenesis and human cancer by Nowell and Hungerford in 1960 (the Ph1 chromosome40), and the description of a signature chromosomal change in CML by Nowell and Hungerford in 1960 (the Ph1 chromosome40) provided a cornucopia for new genes involved in malignant transformation. The clinical success of a rationally designed kinase inhibitor against tumors bearing aberrant kinases39 is the first example of such signaling therapy.

Table 1. Disorders of HGF or HGF receptor expression

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Cytokine/Receptor</th>
<th>HGF/HGFR* Excess</th>
<th>HGF/HGFR Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial erythropoiesis</td>
<td>Epo Rβ1</td>
<td>Pulmonary alveolar proteinosis</td>
<td>GM-CSF Rγ1</td>
</tr>
<tr>
<td>Familial thrombocytosis</td>
<td>Tpoγ2</td>
<td>Amegakaryocytic thrombocytopenia</td>
<td>Tpo Rγ2</td>
</tr>
<tr>
<td>Familial mastocytosis</td>
<td>c-kit receptorα9</td>
<td>Osteoporosis</td>
<td>M-CSF Rδ1</td>
</tr>
</tbody>
</table>

*HGF/HGFR indicates human growth factor/human growth factor receptor.
Table 2. Genetic changes in some common hematologic malignancies

<table>
<thead>
<tr>
<th>Disease</th>
<th>Chromosomal Change</th>
<th>Altered Gene</th>
<th>Putative Mechanism of Oncogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>t(9;22)</td>
<td>BCR and ABL</td>
<td>Altered protein kinase</td>
</tr>
<tr>
<td>Acute promyelocytic leukemia</td>
<td>t(15;17)</td>
<td>PML-RAR</td>
<td>Failed differentiation</td>
</tr>
<tr>
<td>Acute myelogenous leukemia-M2</td>
<td>t(8;21)</td>
<td>AML1</td>
<td>Failed differentiation</td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
<td>t(8;14)</td>
<td>C-MYC</td>
<td>Activated transcription</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>t(14;18)</td>
<td>BCL-2</td>
<td>Reduced apoptosis</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia and non-Hodgkin lymphoma</td>
<td>t(11;14)</td>
<td>CCND1 (cyclinD)</td>
<td>Enhanced cell cycling</td>
</tr>
</tbody>
</table>

Programmed cell death

Several forms of cell death have been recognized since the 19th century. In addition to necrosis induced by physical agents, some cells die in a systematic process characterized by nuclear condensation, internucleosomal DNA breakdown, and organized cellular proteolysis. Although initially thought to represent cellular senescence, this form of programmed cell death, termed apoptosis by Wyllie and Kerr, is a developmentally regulated and reactive process that accounts for the removal of surplus, aged, or injured cells. Our understanding of the mechanisms responsible for programmed cell death is growing tremendously, in large measure due to insights provided by the study of normal and malignant hematopoiesis.

Apoptosis plays a fundamental role in tissue homeostasis, responsible for the removal of a tadpole’s tail and the developmental elimination of a specific 131 of the 1090 somatic cells of the nematode Caenorhabditis elegans. As applied to hematology, programmed cell death is responsible for the purging of autoreactive clones of lymphocytes, which reduces the numbers of activated lymphocytes and phagocytes following an inflammatory response, accounts for reduced levels of erythropoiesis upon growth factor withdrawal, and is a mechanism by which chemotherapeutic agents kill malignant cells. The description of the t(14;18) in follicular lymphomas and the cloning of the fusion gene by several groups in 1985-87 led to the identification of BCL-2, the fusion partner of the heavy-chain immunoglobulin locus in these cells. Approximately 80% of follicular and 20% of diffuse lymphomas bear the signature translocation. The discovery in 1990 that Bcl-2 protein blocks programmed cell death led to the concept that inadequate programmed cell death contributes to malignancy and provided insights into its molecular basis. Bcl-2 localizes to the inner mitochondrial membrane, focusing attention on the role of that organelle and one of its constituent proteins, cytochrome c, on triggering a cascade of protease mediators of programmed cell death.

Integrins

Cell-matrix and cell-cell interactions provide the adhesion necessary for maintenance of proper tissue architecture. Moreover, in addition to soluble mediators of cell signaling, direct cell-cell contact is also a critical mode of intercellular communication. Among the many cellular adhesion systems that have been studied, blood cell interactions with the endothelium and other blood cells have provided valuable and unique insights into the biology of inflammation and stem cell trafficking. The study of platelet-platelet interactions, however, holds a special place in integrin biology; this field can boast the first example of the successful manipulation of cell adhesion for therapeutic benefit.

In 1918 Edward Glanzmann reported on patients who displayed excessive mucocutaneous bleeding but had normal platelet counts. Although Glanzmann was likely studying multiple functional platelet disorders, subsequent investigations using allogeneic anti-platelet antibodies and platelet membrane biochemistry revealed that the bleeding diathesis bearing his name is due to defective platelet fibrinogen binding. The predominant platelet fibrinogen receptor was identified as glycoprotein IIb/IIIa (now termed integrin αIIb/β3 by aficionados) and is a member of the growing family of heterodimeric molecules known as integrins, vital for cell-cell and cell-matrix interactions. The binding between αIIb/β3 and fibrinogen is probably the best-studied interaction between an integrin and its ligand, and its failure in Glanzmann’s thrombasthenia serves as the founding member of diseases of intercellular interaction. The critical binding site, defined by the Arg-Gly-Asp interaction. The critical role played by the hematology community in understanding these fundamental genetic contributions to normal and malignant cellular development should not be underestimated.
tiple factors conspire to generate the ulcerating atherosclerotic plaque, it is the hemostatic system that initiates the final thrombotic event. Occlusive thrombus formation begins with the adhesion of blood platelets to extracellular molecules present in the plaque, including collagen, von Willebrand factor, and tissue factor-generated fibrin. It is unusual for this initial layer of platelets to occlude the vascular lumen; rather, platelet adhesion begets platelet activation, which in turn results in $\alpha_{\text{IIb}}/\beta_3$ and fibrinogen-mediated platelet aggregation. This latter event provides a fait accompli. Studies designed to understand the molecular basis for this series of events have been extremely fruitful. Engagement of any number of platelet receptors (collagen, adenosine diphosphate, fibrinogen, von Willebrand factor) results in activation of multiple intracellular signaling pathways. Much has been learned of the kinases and second messengers responsible for this process. Ultimately, effector molecules are recruited to the cytoplasmic domains of other integrins (including $\alpha_{\text{IIb}}/\beta_3$), altering their extracellular conformation and yielding a high-affinity ligand binding site. Unfortunately, not all of the details of such outside-in and inside-out signaling are presently in place. Nevertheless, it is clear that blockade of integrin function, particularly that responsible for platelet aggregation, should do much to alleviate thrombotic vascular disease and has been an important goal in vascular biology. Two molecular approaches to this problem have recently yielded major dividends.

Using a neutralizing murine monoclonal antibody to human $\alpha_{\text{IIb}}/\beta_3$, Coller and colleagues showed that platelet adhesion and aggregation could be reduced both in vitro and in vivo. Clinical trials of a humanized fragment of this reagent have shown it reduces the incidence of restenosis following coronary angioplasty. In addition, using an understanding of the binding properties of fibrinogen and $\alpha_{\text{IIb}}/\beta_3$, several small-molecule reagents have been developed to interfere with this aspect of platelet function. These agents have also been clinically tested and found to reduce thrombotic events in a number of pathologic settings. The rational use of our understanding of platelet integrin function provides the first example of novel molecular approaches to cell adhesion therapy. Based on this paradigm, intervention in other cell-cell interactions could play an important role in preventing the metastatic spread of cancer by interfering with tumor angiogenesis (eg, blockade of integrin $\alpha_5/\beta_1$); ischemia-reperfusion injuries in shock, stroke, or myocardial infarction (eg, blockade of $\beta_2$ integrins); or the pathologic airway changes induced by inflammatory cell infiltration in asthma (eg, inhibition of $\alpha_5/\beta_1$ integrin). Clinical trials of anti-integrin reagents are already under way in each of these settings. Once again, hematology has provided the critical precedents in a field that stretches across essentially all disciplines of medicine.

Blood coagulation

Hemophilia, perhaps more than any other hematologic disorder, decorates the history of Western culture. The disease was almost certainly recognized in the second century AD: The Talmud describes the decision of Rabbi Judah to withhold circumcision from the son of a woman who had 3 previous sons bleed to death following the procedure. However, Queen Victoria of England began the most colorful chapter of hematology in Western history. An obligate carrier of hemophilia A, Queen Victoria ultimately passed the disease to about 20 people, including the only son of Nicholas and Alexandra (Victoria’s granddaughter) of Russia. It has been argued that the Bolshevik Revolution in 1917 in Russia might never have taken place if not for the preoccupation of the czar and his family with their ailing son, Alexis.

John Conrad Otto established the genetics of hemophilia in 1803, but the biochemistry was not worked out until the 1950s, when hemophilia A and B were distinguished based on complementation of coagulation tests from different families. Although the discovery of cryoprecipitate in the 1960s by Judith Poole led to the first major therapeutic advances for hemophilia A, the purification and cloning of coagulation factors VIII and IX in the 1980s provided purified and now recombinant products for specific therapy and led to characterization of the mutations that cause the human disorders. These studies have given us new examples of disease-causing mutations such as an intron-based genetic inversion. Equally heroic plasma purifications led to the cloning and characterization of the coagulation factors, naturally occurring anticoagulant and fibrinolytic factors involved in normal and pathologic hemostasis. These efforts have provided important new clinical insights; mutations of several clotting and anticoagulant proteins lead to hypercoagulable states, and the use of fibrinolytic agents in patients with acute coronary and cerebral thrombosis has improved survival. The study of coagulation factor biosynthesis has also provided important new insights into the processing necessary for protein secretion; evaluation of patients with combined factor V and VIII deficiency has revealed a chaperone-type molecule necessary for secretion of the 2 structurally related proteins. Detailed study of the structure-function relationships of the coagulation proteins has led to a better understanding of the kinetics of multienzyme complexes, and hemophilia B has provided medicine with one of the few examples of successful gene therapy in large animal models of human disease.

From the foregoing examples, it is clear that our molecular understanding of hematologic disorders grows daily and that its clinical rewards are improved diagnostic, prognostic, and therapeutic approaches to patient care. Although these are examples of how the study of blood and its disorders on a molecular level has advanced both basic science and clinical medicine, an equally impressive list of advances can be compiled for work in cell biology. Elucidation of the ABO and Rh erythrocyte antigenic systems allows virtually unfettered red cell transfusion therapy—yielding revolutions in surgery, the treatment of trauma, and the care of patients with chronic anemias. Advances in chemotherapy, stem cell biology, transplantation, and immunology are only part of the long list of accomplishments based on hematologic investigation begun in the second millennium and that will undoubtably expand greatly in the third. The editorial board of Blood looks forward to our role of reporting, highlighting, and celebrating these accomplishments in the basic and clinical aspects of hematology.

Kenneth Kaushansky, MD
Editor-in-Chief
Seattle, WA

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